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FOR THE  
WATER QUALITY/BIOLOGICAL  
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PROCEDURES MANUAL  
FOR THE  
WATER QUALITY/BIOLOGICAL  
MONITORING AND  
EVALUATION PROGRAM

Prepared for CDIF

by

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Water Quality/Biological Monitoring Surveillance Program Procedures Manual  
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## ABSTRACT

This topical report is a revised version of the draft interim progress report (MERDI No. 3-DOE-MHD-12) submitted to the U.S. Department of Energy on December 1, 1977. The major difference is the presently increased intelligibility arising from better organization. This re-organization allows the reader immediate access, at several levels of technicality, to specific portions of interest.

Terrestrial and aquatic site selection procedures and rationale, plus descriptions of the approximate sampling locations and methodology, are given in the Introduction. Appendix A presents, discusses, and documents procedures for 1) vegetation inventory and community analysis, 2) collection and chemical analysis of both flora and moss ball samplers (for monitoring the chemical composition of dustfall and precipitation), and 3) the collection and physiochemical characterization of soils gathered at the given sites. Appendix B presents both standard and tentative methods for monitoring the following aquatic factors: 1) microbial populations, 2) periphyton communities, including their trace element content, 3) macrophytic species, 4) macroinvertebrate/vertebrate collections, and 5) the physiochemical characterization of surface and ground waters acquired at the sampling sites. Appendix C elaborates on the reduction of raw data into a form compatible with the testing of statistical inference.



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## AMENDMENT(S) AND ERRATA

- Appendix A2, p.3      The protective fence housing each moss sampler unit will be cylindrical, not triangular as depicted in Figure A2-2.
- Appendix A3, p.4      The one square meter ( $1\text{m}^2$ ) Daubenmire frame to be utilized in this study forms a square, not a rectangle as depicted in Figure A3-2. The ten-unit box on the extreme right in this figure represents the 20 x 50 cm microplot sections of the quadrat.
- Appendix A4, p.1      The sentence appearing on lines one and two should be amended to read as follows:
- "The harvest periods will occur as follows:  
a "cool season" in early June, a "peak season"  
in the latter half of July, and a "late season"  
completed by mid-September."



## Water Quality/Biological Monitoring and Evaluation Program

### I. INTRODUCTION

#### A. Statement of the Problem

The MHD process involves elevated operating temperatures (approximately 2600°C) and the presence of potassium compounds in its exhaust stream. The thermal influence on in-process chemistry and kinetics creates uncertainties of both exhaust gas composition (e.g., sulfur and/or nitrogen oxide levels) and partitioning\* of those trace elements in the fuel source. Thus, the environmental impacts and biological responses resulting from these emissions are largely undetermined despite continuing improvements in stack sampling methods and fly ash characterization.<sup>1</sup>

The design of a sensitive biological baseline surveillance program for the CDIF must overcome three problem areas:

1. The uncertainty of the total trace element emission identities, levels, and specific form of each member of the suite (e.g., elemental rather than as an oxide or chloride compound).
2. The insufficiency of existing meteorological data to produce an accurate model of the various air flow patterns within the study basin (especially in modeling the plume dispersion pattern).<sup>2</sup> This problem may be compounded by the possible wide fall-out range of the CDIF's hypothesized small particles.<sup>3</sup>
3. The difficulty in locating and selecting sampling areas that have not experienced the intense impact of past and continuing mining-milling-

---

\* The process of trace element separation into various waste-stream components (i.e., bottom slag, precipitator ash, fly ash) according to their individual physiochemical properties. For example, the volatile element mercury is released nearly completely via the fly ash phase.



smelting or poor grazing practices. (The accurate prediction and utilization of sites that will remain relatively untouched by future impacts--of whatever nature--also must be considered.)

## B. Objective and Scope of Work

The objective of the CDIF surveillance program is to provide the necessary initial data base against which environmental effects from facility construction and operation can be compared and evaluated. It appears the quantification of direct (CDIF-related) and indirect (non-CDIF-related) impacts on the biological communities present in the Butte valley can be separated after the first few years of data gathering and subsequent statistical analysis. This information on CDIF effects then could be applied, with modifications, to an improved surveillance design applicable to larger scale MHD facilities, regardless of location.

Two previous reports<sup>4,5</sup> document the mitigation of the above problems which influence sampling design:

1. The rationale for selection of those first priority trace elements to be monitored in the ambient air, ground and surface waters, soils, vegetation, and animals present in the CDIF study area.
2. The rationale for selection of particular areas that, because of hypothesized influences of meteorology and land form, probably would be affected by CDIF emissions.
3. The preliminary definition of sample site selection criteria.

An additional report<sup>6</sup> discusses the preliminary selection of sampling parameters for both terrestrial and aquatic ecosystems and proposed methods of measurement to meet the above objectives.

The present report documents those techniques utilized by the Water Quality/Biological Monitoring and Evaluation Program (WQ/B) staff to produce



a "minimum but adequate" terrestrial-aquatic monitoring program, with emphasis placed on trace element concentration variance within and between the selected ecosystems over time. (The resulting data from the CDIF impacted vs. background sites then can be analyzed statistically--e.g., via analysis of variances--to judge the relative, intersite emission impact.)

## II. TERRESTRIAL PROCEDURES

### A. Design Phase

Given the uncertainty of an a priori estimation of CDIF impact intensity in the Butte valley, three assumptions were made at the outset of the study design:

1. If the CDIF emissions have an impact on the biological communities surrounding the facility, it will be adverse and chronic in nature.<sup>7</sup> The potential impact will be localized largely to within a several mile radius of operations. It is judged that prior acute anthropogenic--man-made--effects (e.g., arising from the Meaderville mining-smelting complex) already have determined the general composition of the valley's biological communities, including vegetation patterns.
2. Initial methodology will follow the general patterns applicable to the study of the environmental impacts of the conventional coal-fired systems.<sup>10, 11, 12</sup> However, modifications will be made to allow for the past impacts of human activities.
3. Monitoring of biological response to the potential trace elements loading on the surrounding CDIF area will require supplemental measurement of the structure (components) and function (inter-relationships) of the existing ecosystems.<sup>13</sup>



The evaluation of these assumptions requires a probabilistic statement that relies on sample randomization and replication. Furthermore, a valid estimation of the experimental error for each measured environmental characteristic must be provided through calculation. If the claim that CDIF operations have significant environmental impact is rejected, while in reality being true, then the error could contribute to inadequate MHD ETF pollution control design. At worst, it could delay the overall technology development program. This program design, given in detailed form in Appendix A1, incorporates randomized sampling (where possible) and periodic replication of analytical results. The certainty of ultimately rejecting or failing to reject the hypothesis of significant impact in a correct manner will be set at the 95 percent confidence interval.

Given the area's ecological variability, the initial sampling design will delineate the valley according to three impact categories, as shown in Figure 1. This designation assumes that the intensity of past and present anthropogenic impacts has been spread unevenly (in gradient form) throughout the study area. The statistical means of testing the procedure's validity can be found in Appendix A1.

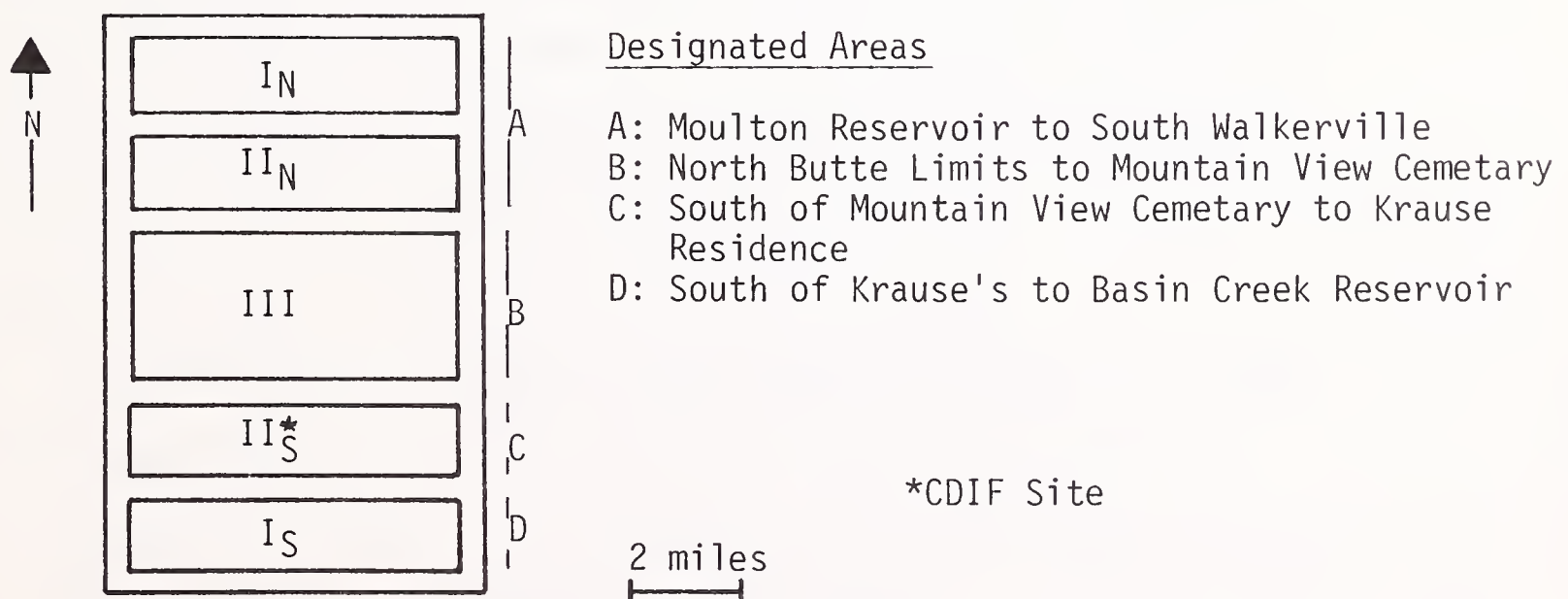


Figure 1.--Pre-Operational CDIF Impact Area Categories with Geographic Delineations (Category I is least impacted.)



This method stratifies the hypothetically impacted area into more uniform subareas. This approach contributes to the control of variability of the values for a given sampled parameter, e.g., soil lead levels; it also assures greater precision in evaluating potential CDIF impacts in the presence of non-direct pollution factors. At least three sampling periods will be utilized to test the validity of this model, via analysis of variance. Appendix A1 contains details of collection sites, periods, and statistical techniques.

Prior to implementation of the intensified field program for the CDIF area (Impact Category IIs, Area C), the following steps were taken:

1. Literature describing the study area's geographic characteristics plus large-scale (1:25,000) infrared and true color aerial photographs were acquired and evaluated. This information was converted into overlay maps, e.g., topographic influences on plant community patterns, to delineate major habitat types. (These habitat types represent areas which have defined ecological characteristics throughout their confines.)
2. Areas of past and present intense human impact were located and eliminated during the selection process in order to avoid gathering dubious or spurious baseline data. This generalized screening also utilized the preliminary, hypothetical CDIF emission impact areas determined by the air quality-meteorology task group. These approximate regions are shown in Figure 2 on the following page.
3. The application of site-specific screening criteria within the generalized impact areas (see Figure 2) included the following:
  - Emphasis on slopes facing the CDIF at elevations below the most intense autumn temperature-inversion height,



- The consideration of duration and scale (plume mass and volume) of CDIF emissions,
- All-season accessibility, with emphasis on locations adjacent to air or water quality monitoring stations, and
- The best possible staff evaluation of the intensity of past, present, and projected disturbances at any given candidate area.

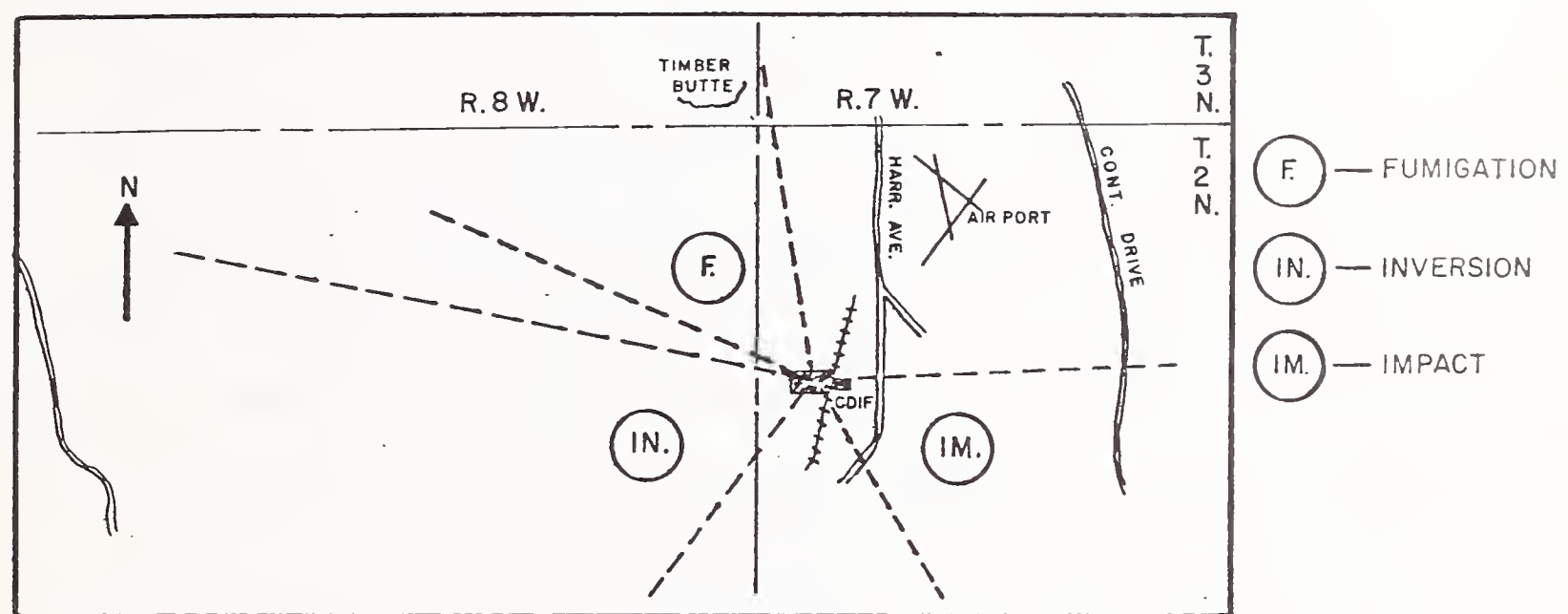


Figure 2.--General Delineation of CDIF Emission Impacts

4. Those areas that satisfied the above criteria and passed field inspection were researched for land ownership. This research was followed by acquiring legal site access.

#### B. Implementation Phase

The initiation of field activities, followed by implementation of the proposed field sampling and laboratory methodology, will proceed in the following manner:

1. Sampling exclosure sites (a protected enclosure) and static (Sphagnum species) moss air quality monitoring sites will be selected:

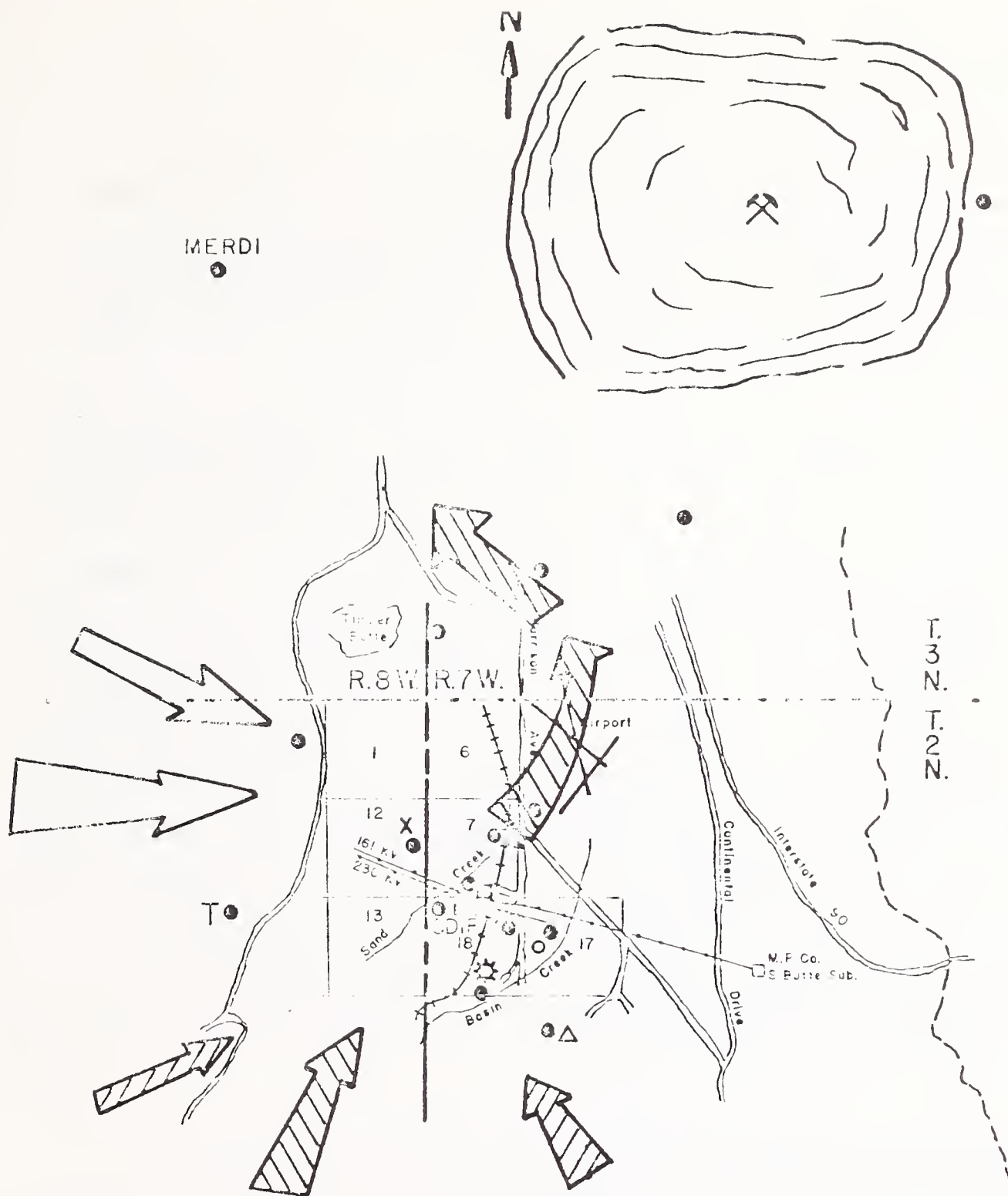


- The exclosures will test the hypothesis that CDIF emissions will be limited largely to Area C. The static samplers will aid in the separation of direct vs. non-direct pollution impacts, thus allowing more effective evaluation of the environmental effects arising from facility operations;
- Presently, five 60 by 40 feet exclosures are being constructed within a radius of about three air-miles of the CDIF; and
- Approximate locations of the exclosures and a number of the moss samplers are given in Figure 3. Construction specifications for both exclosures and moss samplers are given in Appendix A2.

2. The overall methodology for the vegetation studies within each ecologically homogeneous exclosure (i.e., having a specified soil type and plant community throughout) will proceed as follows.

- The enclosed area will be divided into a coded (x, y) matrix composed of 10 x 10 feet macroplots. Prior to each site visitation, six macroplots per exclosure will be selected for sampling via a random number technique. Each macroplot in turn will be sampled intensively by utilizing five one-meter square (1m<sup>2</sup>) quadrats.
- The plant community composition and diversity measurements per quadrat will be accomplished as described in Appendix A3. The combined results of the plant inventory and community diversity calculations should allow evaluation of CDIF effects on the baseline complexity of each of the potentially impacted plant communities (i.e., whether or not the elimination of certain species, with consequent increase in community instability, has occurred over time).
- A plant community burdened with sulfur dioxide<sup>14</sup> and/or elevated trace element levels<sup>15</sup> often responds with a reduction in community

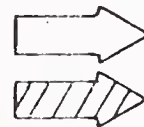




### LEGEND

- CDIF "NORTH" EXCLOSURE
- GOZDEN'S "NO. 2" EXCLOSURE
- ☆ CDIF "SOUTH" EXCLOSURE
- X BALLARD'S EXCLOSURE
- △ PAXSON'S EXCLOSURE
- T "TROUGH" EXCLOSURE (TENTATIVE)
- ▲ "HARRISON AVE." EXCLOSURE (TENTATIVE)
- MOSS SAMPLER STATIONS (PARTIAL DISPLAY)

### PREDOMINATE WIND DIRECTION (S)



DIURNAL

NOCTURNAL

### NOTE:

SIZE OF VECTOR A  
FUNCTION OF FREQUENCY

## CDIF SITE S. OF BUTTE

Figure 3.--Relationship between  
closure sites location  
and predominate wind patterns.

REFERENCE: (Butte Ranger District)  
1968



productivity. This possible effect on the CDIF environs will be considered through the estimation of net community above-ground fiber (biomass) production. The details of the proposed harvest method are given in Appendix A4.

- The determination of plant cover per species represents a quantification of the degree of surface area, or volume, utilized by each member of a given plant community. Analysis of this data, largely on an inter-exclosure basis over the next five years, might indicate changes in ecosystem structure as a result of CDIF emissions.

Methodology for measuring this parameter is given in Appendix A5.

3. As stated in the Introduction, CDIF-caused trace element accumulation, transfer through, and fate in a given ecosystem may or may not be biologically significant. The test of biological significance of the potential trace element burdening in the plant community will be approached as follows.

- At each exclosure-sampling period, the apparently dominant and known elemental "absorber/accumulator" species (e.g., the two-grooved Milkvetch, Astragalus bisculcatus (Hook.) Gray; for selenium) will be collected and stored as described in Appendix A6.1.
- Rather than initially attempting to develop new analytical methods, official or standard approaches will be used in the determinative step. Priority I element concentrations will be analyzed: fluorine, zinc, cadmium, lead, nickel, mercury, arsenic, and selenium. Plant sulfur content will be determined on an intermittent basis. The moss samplers will be analyzed for the above elements plus potassium and beryllium. Gross measurements of alpha-type radioactivity levels for all collected plant materials also are contemplated. Details of



the preparation and analytical procedures for the above elements are described in Appendix A6.2.

4. Upon completion of the plant-related field efforts (at a given exclosure), the following soil-related methodology will be applied in a stepwise manner:

- The soil profile underlying those areas selected for the biomass collection will be sampled at two depth intervals, 0 to 5 cm and 15 to 20 cm below surface grade. At the first interval, a stainless steel coring device will be used for sampling, while the second interval will require cored extraction from a soil pit. Details of the soil profile characterization, sampling, and storage methods are given in Appendix A7.1.
- Initially, total (acid-extractable) Priority I trace ion levels will be determined on both field season samples plus grab samples (within the exclosures) from late April to late October; these "off-season" samples will be used to evaluate seasonal variability for the given trace ion suite.
- The input of detailed stack trace element characteristics will allow the reordering of trace analysis priorities, if necessary, before CDIF start up. The determination of bioavailable (organic solvent-extractable) levels also will be initiated then. These estimates of bioavailability will be determined from samples gathered at the biomass collection sites. Analytical details for the measured soil characteristics, including trace elements, are given in Appendix A7.2.
- Finally, the determination and correlation of pH (acidity/alkalinity) and moisture content data with both total and bioavailable trace



ion data between exclosures and over time would allow

- i) An estimate of relative intersite impact arising from CDIF activities when compared to baseline data,
- ii) A first-order approximation of the specific form(s) a particular trace element acquires (e.g., soluble ion vs. insoluble compound) in a given soil type, and
- iii) An estimate of inter-element percolation rates and subsequent evaluation of potential groundwater pollution arising from the deposited CDIF particulate fall-out.

### III. AQUATIC PROCEDURES

#### A. Design Phase

The design of the aquatic monitoring program was predicated upon the following assumptions (to be tested):

1. The "worst case" potential<sup>7</sup> of the MHD CDIF trace element emissions will be of a chronic nature, as discussed in Section IIA.
2. The effects of the presumed low-volume, intermittent emissions may cause a readjustment of the trace element abiotic-biotic concentration equilibrium.\* However, no lasting change in the baseline biological community composition probably will occur beyond that arising from the natural seasonal cycling (of relative species' abundance) of those organisms present at a given study site.
3. Overall urban/industrial and suburban housing development will influence the evaluation of CDIF effects on the selected water quality parameters as determined at the given study sites throughout the valley's major drainages.

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\*The trace ion concentrations found in the living (biotic) and non-living (abiotic) components plus the rates of exchange between these respective groups.



4. The fine particulate matter that escapes from the CDIF will be distributed more widely than can be measured by the terrestrial and aquatic monitoring sites located within a three air-mile diameter of the facility. However, it is believed that the most intense MHD impacts will be limited to the above 9.5 square miles area (as discussed in Section IIA).

Prior to implementation of the field program, the following steps were taken:

1. Literature describing the valley's hydrogeology and U.S. Geological Survey topographic maps (7.5- and 15-minute scale) were acquired and evaluated. This information was used to delineate four major drainage patterns: three "impacted" ones with a five-mile radius of the CDIF and a northern "control" area. The delineation of these drainages is given in Figure 4.
2. The generally feasible areas were screened using the previously developed site selection criteria.<sup>5</sup> On-site validation of favorable areas was followed by acquiring access to the chosen surface (running and impounded) water and groundwater (well) monitoring sites.<sup>5</sup>
3. The staff then developed the list of those biological, chemical, and physical parameters that will allow evaluation of anthropogenic stress being imposed on the aquatic ecosystems when monitored before, during, and after CDIF activities.

#### B. Implementation Phase

The field sampling and measurement activities have been in effect since June 1977 and occur on a monthly basis. Presently, only a portion of the proposed parameters are being studied; full implementation of the aquatic program is scheduled for June 1978. The monitored categories are given on the following page.





FIG. 4

## MAJOR DRAINAGE BASIN OF THE STUDY AREA

- WATER SITES
- BASIN CREEK DRAINAGE
- - - BLACKTAIL CREEK DRAINAGE
- . . . SAND CREEK DRAINAGE
- - - - YANKEE DOODLE CREEK DRAINAGE (CONTROL)
- ⊗ CDIF SITE



1. The biological monitoring of the selected sites will include the following six categories:

- microbiological,
- periphyton (i.e., organisms attached to underwater surfaces) via 4-cm<sup>2</sup> scrape,
- periphyton via artificial substrate technique,
- periphyton trace element analysis,
- aquatic vascular plant inventory and vegetational analysis, and
- macroinvertebrate (e.g., insects) and vertebrate collections.

Currently, only the microbiological phase is being implemented through the American Public Health Association's Standard Methods.<sup>16</sup> However, the detailed methodology for the above categories is given in Appendices B1 through B6.

2. At each aquatic site, those physiochemical water quality parameters given in Appendix B7 are, or will be, monitored by standard field and/or laboratory procedures. From the standpoint of evaluating biological response to the abiotic environs, the more critical parameters include ionic conductivity, pH, dissolved oxygen, nitrate-phosphate levels, temperature, and flow rate(s).

#### IV. DATA REDUCTION AND STATISTICAL ANALYSIS

##### A. Descriptive Statistical Treatment

This effort will involve the conversion of raw data into a simplified form capable of testing the given hypotheses (see Section B). For each given, measured variable, the following steps will be taken.

1. The tabulated data will be used to calculate the population parameters and sample statistics (including central variability/dispersion, standard error, coefficient of variability, and skew).



2. The data then will be presented in graphic form (e.g., histograms, mirror-image line graphs, scatter diagrams) most appropriate for the given variable(s).

3. The degree of association and consideration of cause-and-effect relationships between the abiotic and biotic factors will be calculated through correlation and regression techniques, respectively. In addition, the degree of confidence attached to the acceptance of these results also will be computed.

B. Inferential Statistical Treatment

This effort culminates the WQ/B staff's intentions to test the validity of the assumptions made in Sections II and III. The statistical testing of these ideas (by the null and alternative hypotheses) set at the 95 percent confidence interval will allow the staff to decide whether or not the CDIF operations do have a measurable effect on the environs.

Additional technical details regarding this section can be found in Appendix C.



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## APPENDIX A



## APPENDIX A1

### PROGRAM DESIGN--THEORETICAL CONSIDERATIONS

If the validity of the assumptions given in the Introduction (IA) is accepted, then the frequency curve of the data for some widespread contaminant (e.g., lead levels in the  $A_0$  soil zone) might resemble a positively skewed distribution, as graphed below.

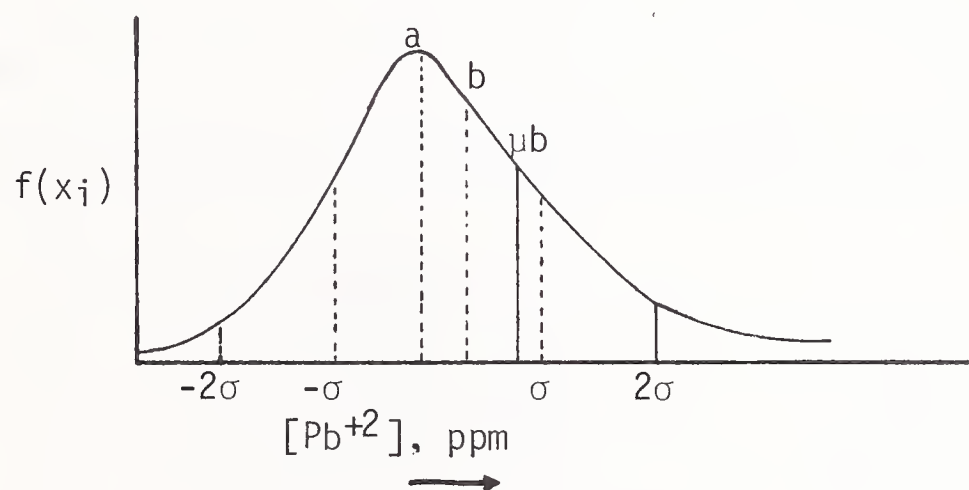


Figure A1-1.--Frequency Curve,  
where a=mode, b=median,  $\mu_b$ =population mean (where n is large, thus  $\bar{x} \approx \mu_b$ )

This distribution reflects the largely ESE to ENE-tending pollution gradient; thus a Gaussian-type distribution, requiring a more even impact pattern, is not expected. A convenient method of testing this stray from standard normality would be to plot the given data on probability paper; this step tentatively is scheduled for all trace elements of Priority I. The proposed relationship between certain intervals of the above curve and the impact category/area term(s) is given on the following page.



| <u>Frequency Interval</u>    | <u>"Equivalent" Impact Category/Area</u>  |
|------------------------------|-------------------------------------------|
| $-2\sigma < Pb \leq -\sigma$ | $I_N$                                     |
| $-\sigma < Pb \leq a$        | $II_N$                                    |
| $a < Pb \leq \mu_b$          | $II_S$                                    |
| $\mu_b < Pb \leq \sigma$     | $III_M$ ("average range")                 |
| $\sigma < Pb \leq 3\sigma$   | $III_E$ ("extremes," e.g., near roadways) |

The possible validity of such stratified impact on the valley will be tested in the following manner:

1. The construction of a sampling grid pattern with sampling points (sites) located from Metzgar-type ("major landmark") maps. The collected data then will be used to construct crude isopleth lines over the grid, as shown below.

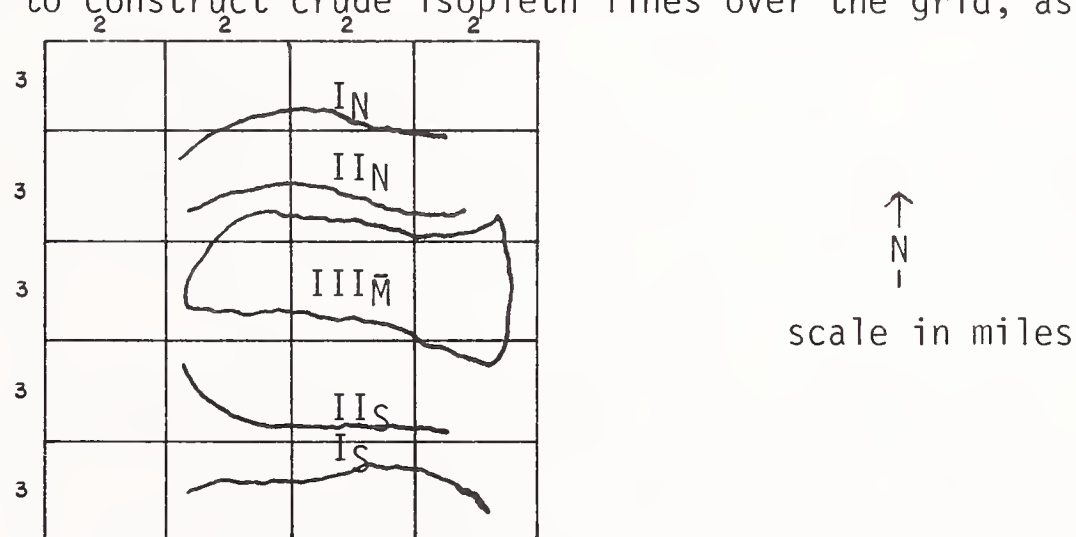


Figure A1-2.--Hypothetical Trace Element Isopleth Distribution Pattern

Such maps will be plotted for several critical, easily measured trace elements (e.g., Cu, As, and possibly Pb). Before proceeding to statistical testing, the individual parameters will be normalized via the z-score technique (i.e.,  $z = (x_i - \bar{x}) / s_x$  for all  $x_i$ ), thus giving a distribution with  $\mu_b = 0$  and  $\sigma \approx 1$ .

2. The normalized data will be graphed by impact category; the possible extremes in results for a given trace element are shown on the following page.



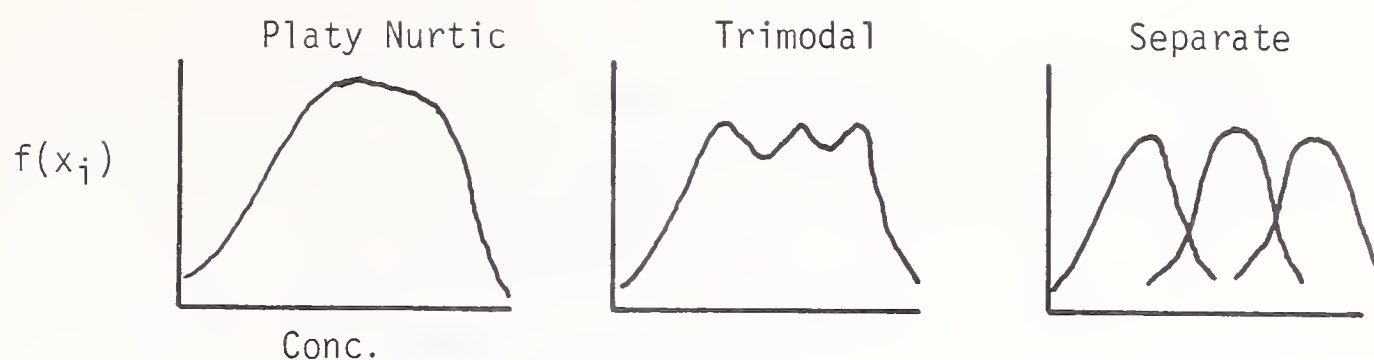


Figure A1-3.--Hypothetical Trace Element Frequency Distributions

The testing of potential impact stratification can be expressed by the following null and alternative hypotheses:

$$H_0 = \sigma^2_{x\mu_b} = 0$$

$$H_a = \sigma^2_{x\mu_b} > 0$$

3. The  $H_0$  states that the baseline population mean,  $\mu_b$ , for the given environmental parameter is the same at each of the impact category areas; any variance observed is due to chance fluctuations about the mean. Graphically, a failure to reject  $H_0$  would imply that the data's distribution is platykurtic to weakly trimodal in nature. The  $H_a$  states that not all means are equal; at least one is different. It should be noted that

a.  $\sigma^2_{x\mu_b}$  will be substituted by  $(\frac{n}{n-1}) s^2_x$ ; thus the experimental confidence interval is not  $\mu_b \pm 1.96 \frac{\sigma_x}{\sqrt{n}}$  but the slightly less accurate  $\bar{x} \pm 1.96 \frac{s_x}{\sqrt{n-1}}$ .

b. The sample size necessary to meet the above 95 percent confidence interval will vary from one monitored parameter to another; however, the general formula and example calculations are given below:

$$\text{sample size } n = \left( \frac{1.96 * \sigma_x}{E} \right)^2$$

where  $\sigma_x$  is a point estimate of the population's standard deviation and  $E$  is the maximum tolerated error per measurement. For example,



- i) for biomass, given  $\sigma_x$  of 25 gms., E of 1.0 gm/m<sup>2</sup> (or ~ 5 percent standard error of  $\bar{x}$  weight per quadrat),  

$$n = \left( \frac{1.96 * 25}{1} \right)^2 = 2401,$$
- ii) for [Mn<sup>+2</sup>], given  $\sigma_x$  of 50 ppm, E of 0.10 ppm,  

$$n = \left( \frac{1.96 * 50}{0.10} \right)^2 = 960,400.$$

Given the ecological variability of the area and budgetary constraints, the initial determination of sample size (n) for all parameters will be done in a somewhat arbitrary manner. It will be assumed that parametric statistical testing can be done on all normalized sample sizes  $\geq 30$ . The calculation of sample sizes capable of meeting the above confidence criteria ( $\alpha = 0.05$ ) will be completed by the end of the FY 78 field season, once  $\bar{x}$ ,  $s_x^2$ , and  $s_x$  have been computed for each environmental variable. At that time,  $\beta$  levels (or probability of committing a type II error by failing to reject a false  $H_0$ ) will be controlled by adjusting (n) for each parameter. A summary of this intent is given below.

Given an  $H_0$

| True | False |                |
|------|-------|----------------|
| OK   | II    | Fail to Reject |
| I    | OK    | Reject         |

4. The actual testing of the impact stratification will be by applying a single-factor analysis of variance (ANOVA), utilizing a one-tailed F test with  $\alpha = 0.05$ . (If the frequency curve appears to be bimodal, then ANOVA will be substituted by Student's t-test with  $\alpha = 0.05$ ). A rejection of the  $H_0$  allows us to believe that a significant difference exists, thus the idea of stratification appears to be valid. If the  $H_0$  fails to be rejected (for example, because of insufficient sample size; see 3b, above), the total



frequency curve will be used for the baseline data distribution for the given variable.

5. The given variable's frequency curve will be compared to the distribution obtained from the operational CDIF period, regardless of the curve's characteristics. Thus, for each trace element, the baseline confidence interval (about the sample mean) will be compared to the interval for each year's operational data to evaluate if any significant ( $\alpha = 0.05$ ) increase in trace element loading has occurred, as shown below.

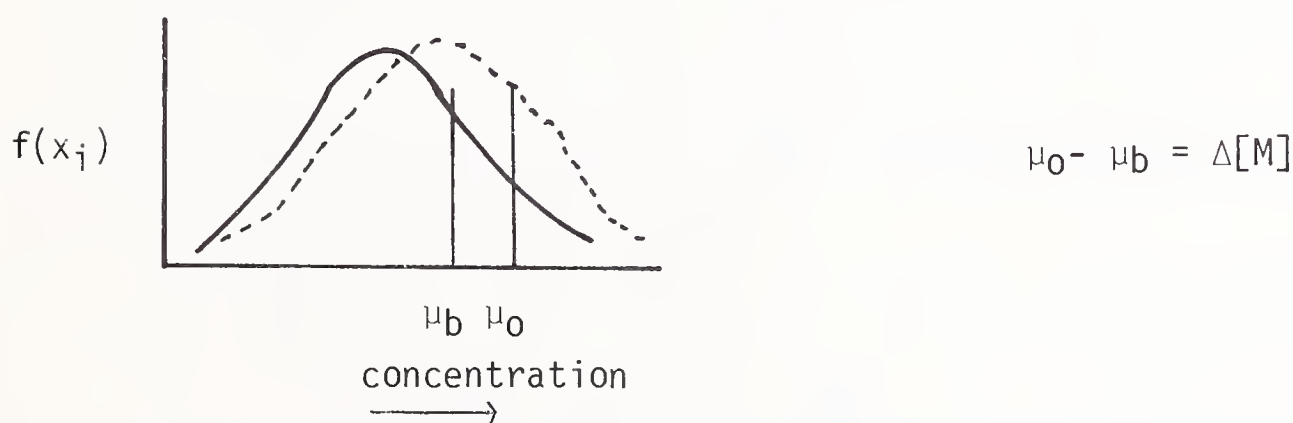


Figure A1-4.--Time Shift of Trace Element Concentration Distribution

This shift from  $\mu_b$  to  $\mu_0$  (or respective sample means) could result if the Impact III area enlarged either uniformly or southward. This expansion, in combination with CDIF emission impacts, would produce an increased  $\mu_0$  for the  $II_S$  (and possibly  $II_N$ ) area.

6. A statistical test of this possibility, especially in Area C (containing the CDIF), would be accomplished by the following technique:

$$H_0: \mu_0 = \mu_b$$

$$H_a: \mu_0 > \mu_b$$

Before monitoring results are available, there is no a priori reason to expect a difference in sample means; therefore, a two-tailed test will be used to check the validity of  $H_0$ . If the value of  $\mu_0$  falls in either rejection region of the distribution [i.e.,  $(\mu_b - 1.96 \frac{\sigma_x}{\sqrt{n}}) > \text{calculated}$



$\mu_0$  or  $(\mu_b + 1.96 \frac{\sigma_x}{n}) < \mu_0]$ ,  $H_0$  will be rejected. If a clear difference appears to exist between  $\mu_0$  and  $\mu_b$ , the following one-side test will be applied.

$$a. \quad z = \frac{\bar{x}_0 - \bar{x}_b}{\sqrt{\frac{(s_0)^2}{n_0} + \frac{(s_b)^2}{n_b}}}$$

b. If  $|z| < 1.96$ , then one will fail to reject  $H_0$  at the 5 percent level of significance.

This alternative also relies on the F-test, where  $H_0$  is rejected if the F value is  $>$  the value of F ( $df_{x_i}$ ,  $df_{error}$ ,  $\alpha < 0.05$ ); i.e., the critical value assigned to the particular test. The one-tailed test evaluating  $H_a$  will utilize the following formula.

$$\mu_D \approx \text{observed } \Delta [M] - 1.64 \sigma_M,$$

$$\text{or } \mu_D \equiv (\bar{x}_0 - \bar{x}_b) - 1.64 (s_0 - s_b)$$

If  $\mu_D > 0$ , then one can be 95 percent confident that the true value of  $\mu_D$  lies to the right of the computed difference interval,  $(\Delta [M] - 1.64 \sigma_M)$ .

An alternative check on  $H_a$  can be obtained from the following formula.

$$d \equiv \Delta M = \frac{\bar{x}_0 - \bar{x}_b}{s_b/\sqrt{n}}$$

A  $d < \bar{x}_b$  would persuade one to fail to reject  $H_a$ .

### Summary

If a real difference in the sample means between impact areas (for a given variable) can be validated, then each area's observed change over time ( $\Delta M_i$  for interval  $i$ ) can be statistically checked at the five percent level of significance. The relative CDIF (direct) vs. non-CDIF (background) impacts then could be evaluated as given on the following page.



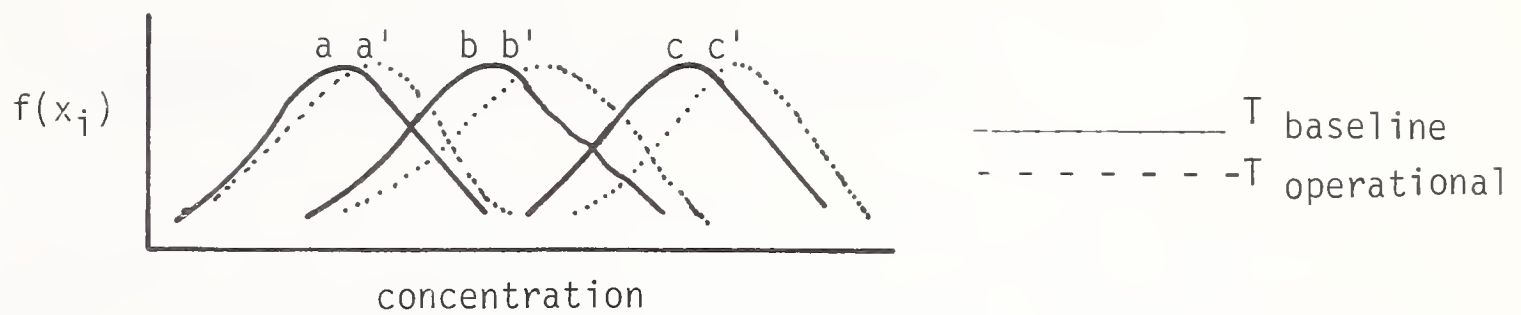


Figure A1-5.--Time Shift of Concentration Distribution for Given Impacts

- An overall frequency curve set for the given variable will be constructed where  $a = \bar{x}$  for impact areas  $I_S$  and  $I_N$ ;  $b = \bar{x}$  for  $II_N$  and  $II_S$ ;  $c = \bar{x}$  for composite area  $III$ .
- The following assumptions are proposed:
  - i) the shift  $(a-a')$  is due to the overall increase in non-CDIF impacts, as is the case for  $(c-c')$ ,
  - ii) the shift  $(b-b')$  will be due to both CDIF and non-CDIF impacts (i.e.,  $|b-b'| \geq |c-c'| \geq |a-a'|$ ).
- The significance of each of the above shifts will be tested at the five percent level of significance using ANOVA techniques. Manipulation of the data to "expand" or "contract" the given impact areas also may serve in the evaluation of the overall geographic impact of the CDIF and thus lead to more effective sampling site locations for a larger scale MHD unit.



## APPENDIX A2

### EXCLOSURE AND MOSS SAMPLER SPECIFICATIONS

The biological monitoring program is composed of two major subtasks: 1) to establish a surveillance system to evaluate potential change in the biological communities present within a three air-mile radius of the CDIF over a period of time and 2) to create an air quality monitoring grid, extending largely through impact areas B and C (see Figure 1 in main text). This latter grid will supplement the formal air quality studies being conducted in the valley by another task group.

At present, MERDI is continuing to prepare terms and conditions for site access leases with a number of valley landowners. This acquisition process will be followed by the construction of protective exclosures and moss samplers having the specifications given below.

#### Static Sphagnum Moss Samplers

The stake-moss static sampling apparatus represents a modified version of the one described by P. Little and M. H. Martin.\* This spherical mass of plant material, enclosed in a wide-mesh nylon net, has excellent water retention and particularly high cation exchange capacity. The analytic coefficient of variation from bags suspended two meters above the earth's surface was found to be within acceptable limits (when compared to accompanying high-volume air sample data) by the above authors. This analytic variability and design validity for the present study's one-meter suspension will be checked for tolerance via the relative standard error on replicate samples.

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\*Little, P. and M. H. Martin, 1974 "Biological Monitoring of Heavy Metal Pollution," Environmental Pollution, Vol. 6, pp. 1-19.



The moss samplers will be replaced on a monthly basis throughout the baseline monitoring period beginning in May 1978. The exposed moss samplers consequently will be analyzed for trace element content via an instrumental method (e.g., flame atomic absorption or graphite furnace techniques).

The resulting data will allow analysis of intersite variability in trace element deposition over the changing seasons.

The sampler construction will require sturdy, chemically inert surfaces; the moss spheres will be protected without undue influence (or interference) on their particle collection abilities. The sampler unit is given below.

#### Legend

- a. 51" upright post,  $\frac{1}{4}$ " diameter hot-rolled steel rod, coated with shellac; to be buried 12" into the given soil profile.
- b. 25" cross wire, plastic-coated No. 10 copper wire, extending 12" on each side from center; to be hooked/looped on each end to support the moss samplers, (c).
- c. Sphagnum species moss balls,  $\approx$  10 grams per ball, contained in a 4 to 6 mm nylon mesh and suspended 6" below the cross wire with light-weight nylon thread.
- d. Packed earth fill, possibly including a small (<5 pound) concrete plug, to anchor and support the upright post.
- e. Longitudinal section of the protective fence (see Figure A2-2).

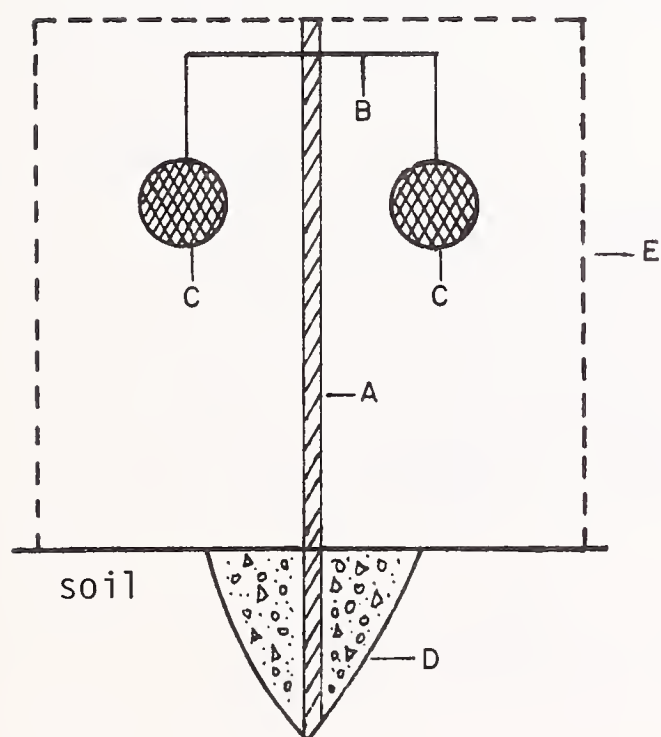


Figure A2-1.--Moss Sampler Design



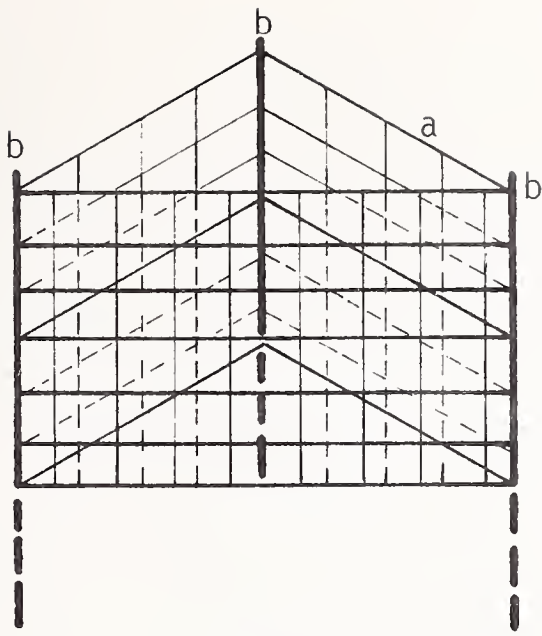


Figure A2-2.--Protective Fence  
Design to House Each Sampler

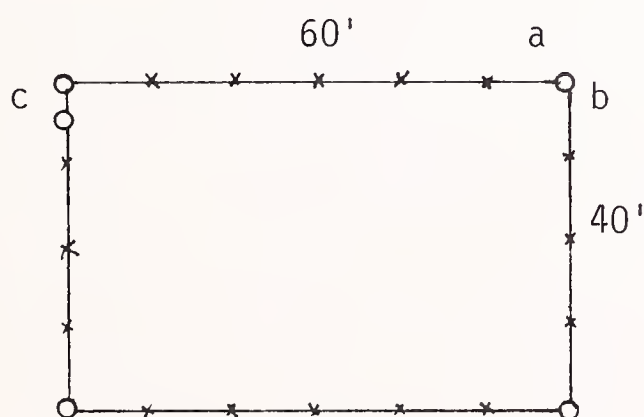
#### Legend

- a. Galvanized field fence, 4" mesh and about three feet in diameter; fence will be coated with shellac.
- b. Stock rod (as in A2-1 above), shellac-coated and secured 12", as above. The fence will be tied to the posts with either shellac-coated galvanized wire or plastic-coated copper wire.

Presently, 20 such samplers will be located throughout impact areas B and C. Another 10 probably will be distributed throughout impact areas A and D (see Figure 1 in text).

#### Exclosures

The exclosures will provide a simple means of excluding domestic and wild grazing species from affecting the monitoring program without creating an undue artificial biological condition within each given enclosed area. The exclosure design is given in Figure A2-3 below.



#### Legend

- a. A 60' x 45' area enclosed by a 47" high stock fence, tied to support posts
- b. Two strands of galvanized barbed wire will be strung above the fence (around the perimeter of the exclosure). This design will allow  $\approx$  55' x 40' (2200 ft<sup>2</sup>) of sampling area, assuming a 2.5 feet margin from the fence on all sides of the exclosure.

Figure A2-3.--Exclosure Design



- b. Steel (rust-protected) fence posts, 80" high with 22" buried in the soil profile. The corner posts will each be anchored with about 0.65 ft<sup>3</sup> of concrete; the other posts, separated at ten-foot intervals around the perimeter, will be set 22" but will not require concrete unless judged necessary to maintain structural stability.
- c. Simple five-foot (maximum) locking swing man-gate; both sides of the gate will have concrete anchored fence posts, as described above.



## APPENDIX A3

### PLANT COMMUNITY COMPOSITION AND DIVERSITY MEASUREMENTS

The plant community composition and diversity measurements will be accomplished in the following manner.

1. The initial visitation to each enclosure will include two phases:
  - The preparation of a field summary of the area's floristic inventory and biogeographical characteristics (e.g., an Artemisia tridentata/Agropyron spicatum shortgrass steppe-shrub synusia growing on an alluvial terrace--0 to 10 percent, SE aspect slopes--in a Basin Creek, gravelly-loam soil type).
  - To supplement the above description, north-south and east-west transects passing through the center of each unit will be completed. Sampling will be done at five-meter ( $\sim 16$  feet) intervals; the vegetation's structure will be recorded in a manner similar to that of Dansereau (Figure A3-1).
2. For each sampling period and enclosure area, one of the  $0.1 \text{ m}^2$  (20 x 50 cm) areas from each quadrat (within each macroplot) will be selected in an arbitrary manner. A hypothetical result of this process is in Figure A3-2.

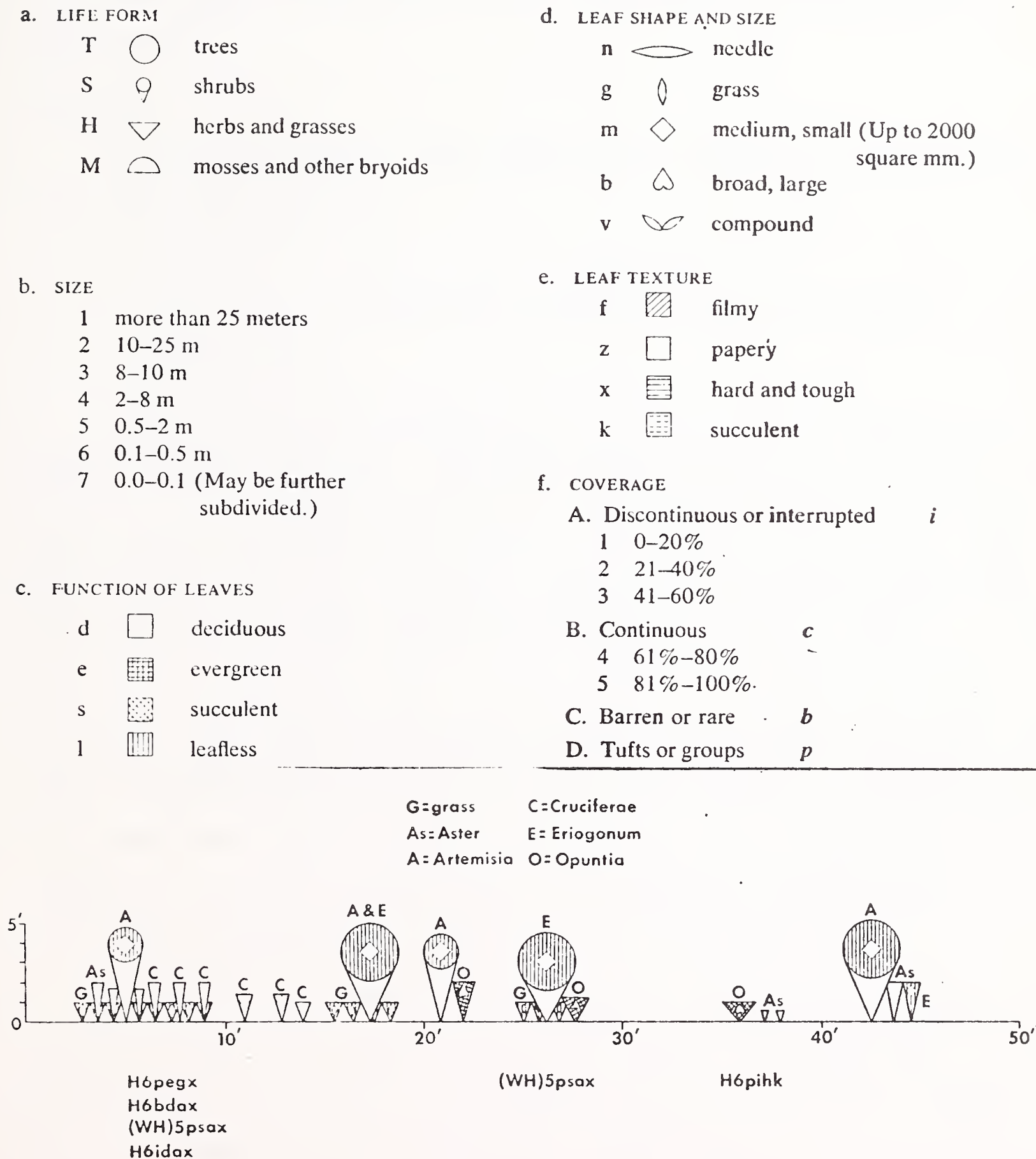
This sample site selection could be performed before leaving for the field. This process selects a total of  $5 \times 0.1 \text{ m}^2 = 0.5 \text{ m}^2$  area from each macroplot or  $6 \times 0.5 \text{ m}^2 = 3.0 \text{ m}^2$  from each enclosure and sampling period. The data will be recorded as shown in Figure A3-3.

The percent frequency of each observed species will be calculated from the above data and then extrapolated to compare intra- and inter-macroplot variation over a given period of time. This long-term trend in potential change from one enclosure to another will be evaluated by changes in the



Figure A3-1

Vegetational Structure Analysis by the Method of P. Dansereau\*



\*Phillips, Edwin A. Field Ecology, American Institute of Biological Sciences, Biological Sciences Curriculum Study, D. C. Heath and Company, Boston, 1964, pp. 33, 45.



calculated Shannon-Weaver diversity index,  $H'$  over time. Furthermore, the raw data also could be utilized to calculate species' density values (i.e., the number of representatives per given species observed within a defined area).

### Discussion

The vegetation study is based upon the two following assumptions about the area's plant communities:

1. They will be subject to a polyclimax scheme, yet
2. They can be delineated into definable plant associations or habitat types.

The sample data must be representative of the given exclosure or ecological response unit (ERU). The cluster sampling technique of Cook and Bonham<sup>1</sup> will allow accomplishment of this requirement. Such a method (also termed simple restricted random-block sampling) involves the delineation or exclosure of an area that represents the potential strata (or stands) existing within the ERU. Thus, the area should be representative of the differences that occur in the somewhat larger areas throughout the given broad land/vegetation classification scheme. The cluster approach differs from traditional stratified sampling in that each exclosure does not necessarily possess (or isolate) some set of delimiting factors (e.g., a soil phase of some given soil type) within the sampled ERU area.

The vegetational inventory data represents a semi-quantitative description of the given study area's floristic pattern, presenting its community appearance (physiognomy), species, and their life forms. Through the use of references on plant pathology, a brief comment on the apparent state of plant community health per ERU will be noted in the inventory, including



- supposed mineral deficiency and/or excess, plus other abiotic stress factors (including air pollution effects) and
- supposed biotic infestations (e.g., dwarf mistletoe on conifer species).

Density (an index of species' abundance) and frequency (an index of species' uniformity per unit area) are important statistics in the analysis of community composition and diversity. The sampling conditions for determining the latter parameter are important since the calculated frequency values per given species set are a function of quadrat size, plant size or mass per species, and their relative spatial distribution.<sup>2</sup>

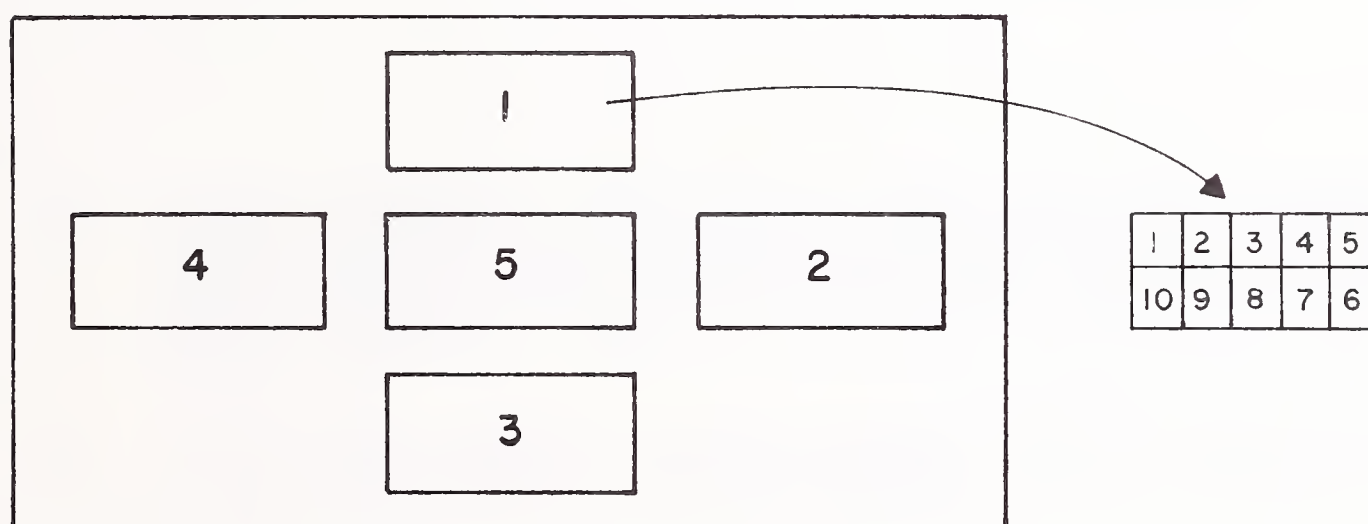


Figure A3-2.--Macroplot and Microplot Designations



Figure A3-3

Production (Field) Data Sheets<sup>(a)</sup> - Format

Study Site (ERU) No. \_\_\_\_\_ Date \_\_\_\_\_

Soil Type \_\_\_\_\_

| SPECIES OBSERVED | Quadrat No. <sup>(b)</sup> |   |   |   |   | Macroplot No. _____ |
|------------------|----------------------------|---|---|---|---|---------------------|
|                  | 1                          | 2 | 3 | 4 | 5 |                     |
| _____            |                            |   |   |   |   |                     |
| _____            |                            |   |   |   |   |                     |
| _____            |                            |   |   |   |   |                     |

(a) Harner, Richard F. and Kenneth R. Porter, Vegetation Study. Twin Creek Coal Project, Lincoln County, Wyoming for Rocky Mountain Energy Company, Dames and Moore Ecological Consulting Service, Denver, Colorado. Report No. 6787-024-14, August 6, 1976. Appendix C.

(b) Include the randomly selected subsample ( $0.1\text{m}^2$ ) frequency and biomass ( $0.2\text{m}^2$ ) areas for each sampled quadrat.



## REFERENCES

1. Wayne C. Cook and Charles D. Bonham, Techniques for Vegetation Measurements and Analysis for a Pre- and Post-Mining Inventory, Colorado State University, Department of Range Science, Range Science Series, No. 28, pp. 45-53, 1977.
2. Rexford Daubenmire, Plant Communities--A Textbook of Plant Synecology, Harper and Row Publishers, New York, New York, pp. 1-35, 1968.



## APPENDIX A4

### BIOMASS DETERMINATION

The harvest periods will be set to coincide with the "cool season" (mid-June) and "warm season" (mid-August) growth maximums; the gathering of plant material during the autumn rainy or "green-up" period in mid-October also is contemplated. During the course of each exclosure sampling period, the following procedure will be applied:

1. One of the five quadrats per given macroplot will be selected in a random manner. From this quadrat, the living and dead aerial tissues (i.e., the given season's growth) will be clipped at ground level using a pair of clean steel scissors. A total 0.2 m<sup>2</sup> area, comprised of the 0.1 m<sup>2</sup> frequency microplot (see Appendix A3) and one other randomly chosen 0.1 m<sup>2</sup> area, will be harvested. The shrub (e.g., Chrysothamnus) species will be sampled by clipping the present year's growth, located largely in the crown region.
2. The contents from each macroplot will be transferred to clean, labeled paper sacks for transport back to the laboratory. The tissues per bag will be placed in individual clean 0.5 to 1.0 liter beakers and dried at 100 ± 5°C for 24 to 36 hours. The samples must be dried quickly to minimize respiratory losses, while only the near-constant weight observed per sample is recorded in the biomass data section.

#### Discussion

As determined by the above method, the biomass data serves as an indicator of surface net primary productivity, expressed as grams of oven-dry plant tissue per m<sup>2</sup>. Analysis of the data, largely on an intersite basis over a period of time, might indicate changes in general ecosystem function



resulting from CDIF trace element emissions in a manner similar to that observed in a Swedish case study (see reference).



## APPENDIX A5

### PLANT COVER (BY SPECIES) DETERMINATION

The plant cover determinations will be accomplished in the following manner:

1. For herbaceous flora  $\sim 14"$  ( $\leq 35.6$  cm) in height, a point-quadrat type method will be utilized. The species present and their estimated area of coverage at the 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 meter marks (along the central axis of each quadrat) will be recorded.
2. A line-intercept type method will be used for estimating shrub coverage by sampling from the five quadrats per macroplot. In both cases (i.e., steps 1 and 2), the observations will be recorded in the manner shown in Figure A5-1. Phenological data will be incorporated with the above information and recorded in a manner similar to that used by E. A. Phillips (see Figure A5-1). The basal areas of the herb species can be determined by either caliper or metric ruler measurement through the diameter of a given grass culm/clump or small forb cluster. The "shadow," or diameter, covered by a downward projection of the foliage's volume will determine an estimate of coverage by the shrub and tall dicot (and monocot) species.

#### Discussion

Plant cover quantifies the degree of surface area (or aerial volume) used by the given plant community per study area, as diagrammed in Figure A5-2.

The determination of this parameter via the line-intercept method is rapid, objective, and relatively accurate; the sampling lines can be placed randomly and replicated to obtain the desired level of precision. The method is not suitable in the case of intermingled vegetation types and/or indistinct community boundaries (see reference); however, the initial selection of the ecological response unit area largely should eliminate this problem.



Figure A5-1

Format for Cover<sup>(a)</sup> and Species Phenology<sup>(b)</sup> Data

Distance<sup>a</sup> (on line-intercept, in meters or centimeters) Spp.obs.

0-1 Agsc. 1.2cm<sup>2</sup>(=); Feid. 7.5cm<sup>2</sup>(?); Phho. 12.5cm<sup>2</sup>(0)

1-2 Agsc. 7.5cm<sup>2</sup>(=); Phho. 10.0cm<sup>2</sup>(0); Ciar 15.5cm<sup>2</sup>(+)

2-3 Agsc. 4.5cm<sup>2</sup>(=); Phho. 8.5cm<sup>2</sup>(0); Ciar 20.5cm<sup>2</sup>(+); Feid 5.0cm<sup>2</sup>(+)

Phenology code<sup>b</sup>

| <u>Symbol</u> | <u>Explanation</u>     |
|---------------|------------------------|
| -             | Floral inactivity      |
| +             | budding                |
| x             | flowering              |
| =             | flowering and fruiting |
| 0             | fruiting               |
| ?             | unknown-uncertain      |

(a) Phillips, Edwin Allen, Methods of Vegetation Study, Holt, Rinchart, and Winston, Inc., New York, New York, 1959.

(b) \_\_\_\_\_, Field Ecology American Institute of Biological Sciences Curriculum Study. D.C. Heath and Company, Boston, 1964, Table VI-4, p.70.



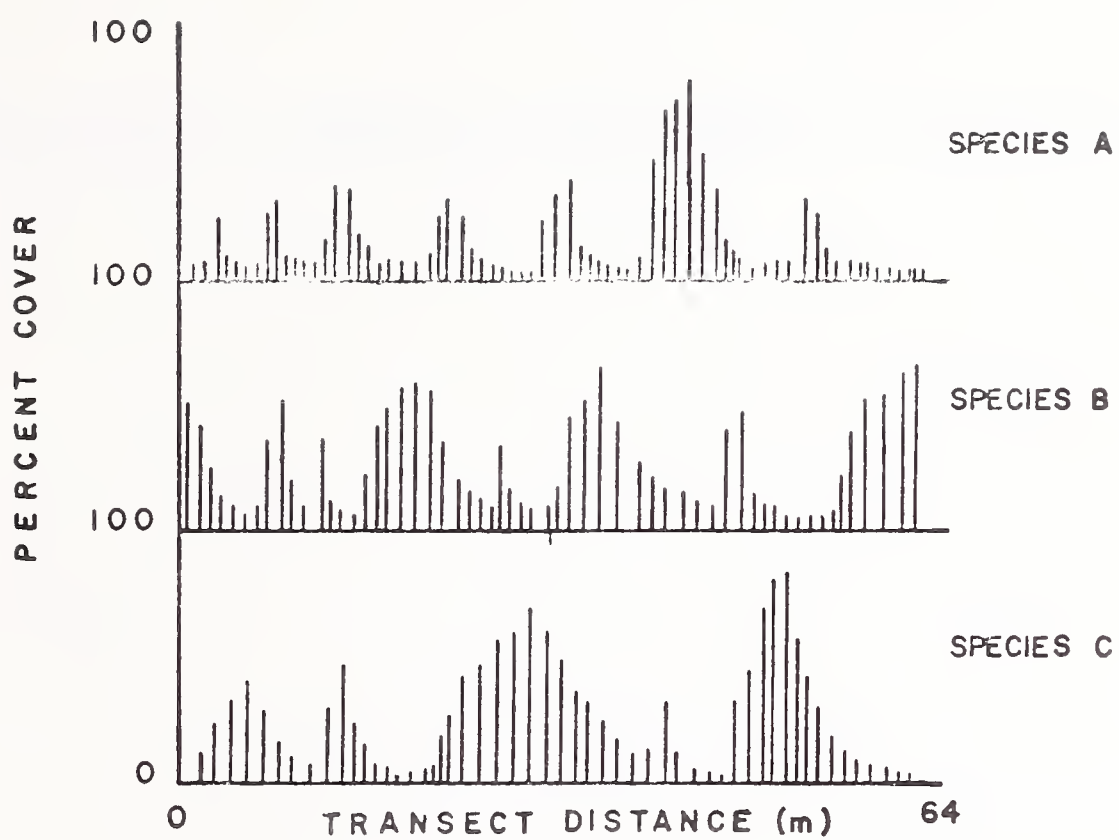


Figure A5-2.--Hypothetical Presentation of the Coverage Data per Exclosure



## REFERENCE

Robert Lee Smith, Ecology and Field Biology, Second Edition, Harper and Row Publishers, New York, New York, 1974.



## APPENDIX A6.1

### VEGETATION--SAMPLING PROCEDURES

The plant species available within a given macroplot will be inspected for the presence of dominant or known elemental "absorber/accumulator" types. The sampling method will proceed as follows:

1. A quadrat will be selected from each macroplot in an arbitrary manner.

Two 0.1 m<sup>2</sup> microplots then will be selected at random; if the previously harvested (biomass) microplot is selected, the process is repeated until two unclipped areas have been obtained.

2. Using clean steel scissors, about six 5-gram (minimum) aliquotes of semi-dry to wet tissue will be collected for each selected species; each aliquot will be

- a. taken from an existing forb cluster or grass culm, and
- b. the same plant organ(s) (e.g., leaves, stems) on all sides of the sampled material will be collected at a predetermined height above ground level (>2 inches--~5 cm) for herbaceous species, at crown level for semi-shrub and shrub species, and 5.5 to 6.5 feet (~1.7 to 2.0 m) for taller shrub and tree species).

3. The tissues of each collected species will be placed in separate, labeled polyethylene bags and sealed for transport back to the laboratory. The samples then will be identified, if necessary, through the use of local flora taxonomic keys and finally frozen (at  $T \leq -15^{\circ}\text{C}$ , ~ 0°F) prior to preparation and analysis.

#### Discussion

The sampled plant material must be gathered in a consistent and uniform manner. Hopefully, the species sampled for trace element determination



accumulate or absorb these elements in a reproducible manner, the tissue concentration being proportional to that level biologically available in the soil medium. The priority placed on the various plant factors considered in the above sampling design are listed in the following table.<sup>1</sup>

Table A6.1-1.--Criteria Involved in Plant-Sampling Design

| <u>Factor</u>        | <u>Relative Importance</u> | <u>Method of Reducing the Factor's Effect</u>                               |
|----------------------|----------------------------|-----------------------------------------------------------------------------|
| Plant Species        | Very Great                 | Orientation-Pilot Survey                                                    |
| Organ Sampled        | Great                      | Orientation-Pilot Survey                                                    |
| Age of Organ         | Significant                | Orientation-Pilot Survey                                                    |
| Root Depth           | Significant                | Concentration Ratios of 2 Elements Per Sample                               |
| pH                   | Fairly Significant         | Concentration Ratios of 2 Elements Per Sample                               |
| Plant Health         | Fairly Significant         | Selection of Healthy Specimens Only                                         |
| Drainage Effects     | Fairly Significant         | Element Ratio (e.g., S:Se Concentration Studies)                            |
| Element Availability | Fairly Significant         | Ratio of Element Concentrations of Same Bioavailability to a Given Species. |

It should be noted that the categories given in the center column of the above table are indeed relative, serving only as a general guide (and varying from one considered element to another) to those factors that can bias the observed results if ignored during the sampling program. It also is of great importance to locate sites at distances removed from the impact of vehicular exhaust emissions since several studies have shown elevated heavy metal concentrations in soils<sup>2</sup> and/or accumulations into the herbivore-trophic level<sup>3,4</sup> in areas adjacent to heavily traveled roadways.



## REFERENCES

1. R. R. Brooks, Geobotany and Biogeochemistry in Mineral Exploration, Harper and Row Publishers, New York, New York, Table 11-10, p.111, 1972.
2. J. W. Lagerwerff and A. W. Specht, "Contamination of Roadside Soil and Vegetation with Cadmium, Nickel, Lead and Zinc," Environmental Science and Technology, 4(7):583-586, 1970.
3. F. E. Giles, S. G. Middleton, and J. G. Gran, "Evidence for the Accumulation of Atmospheric Lead by Insects in Areas of High Traffic Density," Environmental Entomology, 2(2):299-300, 1973.
4. Peter W. Price, Beverley J. Rathche, and David A. Gentry, "Lead in Terrestrial Arthropods: Evidence for Biological Concentration," Environmental Entomology, 3(3):370-372, 1974.



## APPENDIX A6.2

### VEGETATION--CHEMICAL ANALYSIS PROCEDURES

#### Preparation for Analysis

The following procedure will be applied to all non-Sphagnum species plant material before chemical analysis.

1. Approximately 15 to 20 gms of recently thawed tissue per sample pack will be withdrawn arbitrarily; the remaining tissue in the sack will be returned to the freezer. The partially thawed material will be placed in a clean, towel-lined petri dish and chopped into 1 to 2 cm lengths with a clean, 30 percent H<sub>2</sub>O<sub>2</sub>-rinsed sharp razor blade or scalpel. Initially, the total plant will be used (i.e., without concern for isolation of stems from leaves or seed heads) when preparing grass or forb species.
2. The chopped tissue sample then will be placed in a 250 to 800 ml beaker (the size being determined by sample volume), labeled, and dried in a forced-draft oven at  $40 \pm 1^{\circ}\text{C}$  for up to 48 hours.
3. When the samples are dry, they will be removed from the oven and ground to pass through a stainless steel mesh (aperture  $\sim 1$  mm) using an intermediate model Wiley mill. This material will be reground to pass 20 mesh ( $\sim 850$   $\mu\text{m}$ ), collected in clean 50 to 100 ml volume glass or plastic vials. Each sample vial then will be labeled, capped, and stored in a cool, dry place if analysis is imminent. If a considerable analytic backlog occurs, the prepared samples will be stored at  $0 \pm 1^{\circ}\text{C}$ , then dried to constant weight before weighing out an aliquot for digestion.

#### Trace Element Analysis

For each prepared dry sample slated for trace element (Priority I, except fluorine) analysis, the following procedure will be utilized.



1. One to two grams (to the nearest 0.1 mg) on the analytical balance will be weighed out, and the material will be transferred to a clean 500 ml Erlenmeyer flask.
2. Approximately 30 ml of double-distilled, AR-grade, concentrated nitric acid ( $\text{HNO}_3$ ) will be dispensed to each flask; if the sample material is believed to be high in protein, fatty, or resinous substances (e.g., seeds, conifer needles), the flasks should be set under the hood for about  $\frac{1}{2}$ -hour before heat is applied.
3. The prepared flasks and reagent blank(s) will be swirled gently to mix the acid with the plant tissue; the inner walls of the flasks will be washed with a small amount of the above  $\text{HNO}_3$  and placed on a preheated,  $170^\circ\text{C}$  hotplate.
4. The flasks will be allowed to digest gently until the expulsion of the brownish  $\text{NO}_2$  is nearly complete. The heat then will be increased to  $195^\circ$  to  $200^\circ\text{C}$  (taking care to avoid severe bumping or sample spattering) for about  $1\frac{1}{2}$  hours or until the usually straw-colored liquid is reduced to several milliliters volume.
5. The inner walls of the flasks will be rinsed with  $\leq 1$  ml of AR-grade, 30 percent  $\text{H}_2\text{O}_2$  (per flask), then  $\sim 5$  ml AR-grade 60 percent perchloric acid ( $\text{HClO}_4$ ) will be added to each flask, including the blank.
6. The volumes then will be reduced to near dryness at  $200^\circ$  to  $205^\circ\text{C}$  without charring, until dense, white  $\text{HClO}_4$  fumes fill the flasks. Several additional volumes ( $\leq 2$  ml total) of  $\text{H}_2\text{O}_2$  can be added to each flask while swirling to complete the decolorization-oxidation at this stage. (Rapid or explosive oxidation problems resulting from metallic perchlorate(s)-organic matter reactions should not be observed as long as the oxidation process is largely complete before final  $\text{HClO}_4$  fuming.) Heating will be continued until the reappearance of white fumes.



7. A small amount ( $\leq 0.1$  mg) of AR-grade potassium dichromate, when added to the above solutions, will remain green if the solution remains in a reducing condition (i.e., organic matter is still present).<sup>1</sup> If this is the case, a milliliter of  $\text{HClO}_4$  will be added, and heat will be applied until the solution turns bright orange, indicating an oxidized state. For controlling background contamination arising from the salt, the amount added to each flask should be controlled carefully (e.g., dispensed with a scoop calibrated to deliver a known amount of solid per flask).
8. The completed digests and blanks will be removed from the hotplate, and several milliliters of AR-grade (distilled-deionized) water (DI) will be added to each flask. This serves to cool the reactions and dissolve the acid fumes into the digest solution.
9. The cooled digests are filtered preferably through sintered glass disks under light suction. The flasks are rinsed with small amounts of warm DI ( $\leq 2$  ml/flask) into the funnel system. A small number of experimental (non-sample) flasks will be rinsed with warm  $10^{-2}\text{M}$  AR-grade hydrochloric acid (HCL) to evaluate relative (compared to DI) ion-elution efficiencies, thus demonstrating which solvent is most effective in desorbing those ions adhering to the sample's insoluble silica matrix.<sup>1</sup>
10. The filtrates will be collected in 25 ml volumetric flasks and diluted to volume. The analyte (Priority I) concentrations will be determined either by appropriate carbon-cup atomic absorption<sup>2,3,4</sup> or inductively-coupled Argon plasma atomic emission spectroscopy procedures.

#### Trace Element Reconnaissance with Moss Sampler Bags

Tentatively, there will be about 20 sampling sites located near residences at various distances and directions from CDIF operations. However, all such sites will face the CDIF area to assure maximum monitoring sensitivity.



The construction specifications for the samplers are given in Appendix A2. The following procedures describe the preparation and chemical analysis of the moss balls.

1. One gram (to the nearest mg) batches of DI-washed, air-dried ( $45 \pm 2^{\circ}\text{C}$ ) Sphagnum moss will be weighed out using an analytical balance. The moss (e.g., Sphagnum squarrosum) is obtained in bulk from a biological supply company.
2. Each sample will be wetted with a small volume ( $\leq 2$  ml) of DI-water and compressed or molded into near spherical balls  $\sim 4.5$  cm ( $\leq 2$ " ) in diameter; care must be taken to avoid contamination by improper or careless handling during this preparatory step. Four extra balls per monthly lot of 40 will be saved in a frozen state until they can be analyzed for their inherent trace element content.
3. The two balls per site for all sites will be kept in place for a one-month period. At that time, they will be replaced with fresh samplers. The exposed balls will be placed in individual, labeled polyethylene bags, sealed, and transported back to the laboratory's cold storage facilities.
4. Each moss sampler will be removed from its bag, transferred to a 250 ml beaker, and dried in a forced-draft oven at  $40 \pm 1^{\circ}\text{C}$  for about 24 hours. The dried samples will be crushed lightly in a Wiley mill to pass number 10 mesh, and the passed fibers will be collected in labeled, 50 to 100 ml plastic or glass vials. The samples will be stored in a cool, dry place prior to analysis.
5. One gram (to the nearest 0.1 mg) will be weighed from each sampler vial; then the previously given digestion procedure will be utilized.
6. The instrumental step will be the same as given in the general discussion; however, beryllium and potassium will be included in the trace ion (Priority I) suite for analysis.



### Tentative Plant/Moss Sampler Fluorine Analysis<sup>5</sup>

The plant/moss sampler analysis for fluorine analysis will proceed as follows:

1. One-half gram (to the nearest mg) of the dried, ground plant material will be weighed out and transferred to a 35 to 50 ml capacity nickel (or other resistant metal) crucible.
2. The plant material will be mixed with 0.05 gm low-fluorine, AR-grade calcium oxide and slurried with  $\leq 2$  ml DI-water. The samples will be dried and pre-ashed under IR heat lamps for approximately  $1\frac{1}{2}$  to 2 hours. They then will be covered and ashed overnight ( $\sim 12$  hours) at  $600^{\circ}\text{C}$  in a muffle furnace.
3. The cooled contents per crucible will be moistened with DI-water and then dissolved in a minimal volume ( $\leq 1$  ml) of 30 percent  $\text{HClO}_4$ . The individual volumes will be transferred to 100 ml volumetric flasks with small amounts of 50 percent total ionic strength acid buffer (to wash out both crucible and funnel into the given flask), then diluted to volume with this reagent.
4. The fluorine content of each sample is determined with a calibrated Orion<sup>®</sup> fluoride specific-ion electrode system. The analytical results will be interpolated against the results from the known fluorine standards. A 1.0 ml spike of 5 ppm  $\text{F}^-$  solution can be added to those samples initially containing very low fluorine levels.

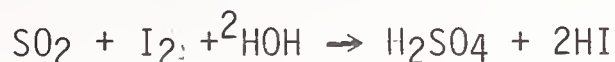
### Tentative Plant/Moss Sampler Sulfur Analysis<sup>5</sup>

The plant/moss sampler analysis for sulfur will proceed as follows:

1. An 0.10 gram portion of the dried, ground sample material will be placed in a ceramic cup and mixed with a 2:1 w/w mixture of elemental tin and iron catalyst.
2. The total mixture will be ignited in an oxygen atmosphere within the sample chamber of a Leco<sup>®</sup> induction furnace.



3. The oxidized sulfur, as SO<sub>2</sub>, bleaches the bluish starch solution in the collection flask by the following chemical reaction:



4. The initial blue hue is maintained in the presence of the evolved SO<sub>2</sub> by titrating with potassium iodate solution, as summarized below.



From the utilized titrant volume, the initial sulfur content (usually expressed in ppm) can be calculated.

### Discussion

The determination of trace element constituents in plant (and soil) material is beset with numerous difficulties, many of which either are realized incompletely or generally unappreciated. An overall sensitivity to these potential sources of error largely can eliminate the gathering of faulty data if precautions necessary for their reduction or elimination are taken. A brief list of the "potential error classes" are listed below.<sup>6</sup>

| <u>Contamination</u>                                                 | <u>Loss of Constituent</u>                                                         | <u>General</u>                                                                                            |
|----------------------------------------------------------------------|------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|
| reagents<br>air-water<br>apparatus-containers<br>atmospheric fallout | absorption/precipitation<br>chelation/ion exchange<br>volatility<br>matrix effects | sampling/storage<br>methods<br>inaccurate standards or<br>calibration problems<br>instrument malfunctions |

Rather than place emphasis on the development of new analytical methods or techniques (at least in the initial phase of the trace element reconnaissance program), official or standard methods will be employed in the determinative step.

The compiled analytical results will be interpreted statistically, including an analysis of variance at the following levels of hierarchical sampling organization; namely, at the categories of impact region, exclosure(s), macroplots per exclosure, and intraplot variability between quadrats.



Details of the requisite statistical design and calculation methodology can be found in Appendix A1.



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## APPENDIX A7.1

### SOILS--SAMPLING AND CHARACTERIZATION PROCEDURES

Once in the field, each exclosure's physiography will be noted and related to the known facts of its geologic history. A number of preliminary auger-borings should be made to validate the supposed soil type (as intimated from the local soil survey maps). Inspection of the cores also should ensure that the selected area is not anomalous (i.e., not showing evidence of recently disturbed soil horizons or contamination by lime, fertilizers, or organic wastes). The sampling method will proceed as follows:

1. From each of the two 0.1 m<sup>2</sup> areas selected per macroplot for the biomass determination (see Appendix A4), an auger will be used to collect at least 20 5-cm long cores (i.e., 0 to 5 cm in the soil profile). The cores will be placed in a labeled, polyethylene bag or whirlpack and sealed. This process will be repeated at each macroplot throughout each exclosure for the designated sampling periods.
2. An arbitrary method will be used to determine which 0.1 m<sup>2</sup> microplot area from the biomass quadrat will be selected for soil pit excavation. The soil pit will be of the form illustrated in Figure A7.1-1.

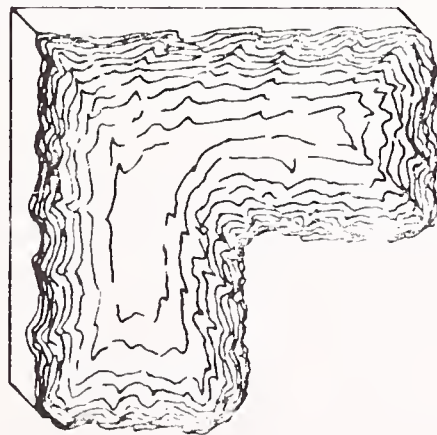


Figure A7.1-1.--Soil Pit Schema for Subsurface Soil Collections



3. The L-shaped pits will be dug with a clean, sharp-edged, garden-type spade. The horizon boundaries will be located (if present), and their depth, structure, texture, color characteristics, and general plant root distribution recorded in the field notes; the format of these records is given in Figure A7.1-2. Care must be taken to keep the ground surface adjacent to the pit ( $\leq 3$  ft.) free from excavated material; this effort should minimize the chance of contaminating the 15 to 20 cm soil depth samples with surface materials.
4. The 15 to 20 cm subsoil samples will be gathered in the same manner as that for the surface coring; furthermore, all soil samples will be maintained in storage at  $0 \pm 2^{\circ}\text{C}$  until prepared for analysis.

#### Discussion

The methods of sampling will be modeled closely after those given by the proper authority<sup>1</sup> or by the USDA Soil Conservation Service field manuals.<sup>2,3</sup> A flat, square-point spade is superior to the post-hole spade excavations<sup>3</sup> in the expected gravelly soils that occur in the general CDIF area; a geologist's hammer or hand pick also will be of use in such dry, rocky soils. A core-type auger is preferred over a screw-type auger in this sort of study since the cored sample will give a more accurate presentation of the given area's soil structure, porosity, and consistency than can the latter type, especially in porous-dry soil types.

It is believed that the 320 surface and the 320 subsurface cores per ERU sampling period will represent sufficient sample mass to minimize the sampling error resulting from the intrinsic variation of values for any given soil property or parameter within any given ecological response unit. Composite subsample or bulk analysis methods will not be applied initially since no estimate of the variance of the mean and, hence the precision with which the



Figure A7.1-2

Format(s) for Soil Description-Characterization<sup>(a)</sup> Data

Ecological Response Unit No. \_\_\_\_\_ Date \_\_\_\_\_

Plot No. \_\_\_\_\_

Soil type \_\_\_\_\_ Soil classification \_\_\_\_\_

Location \_\_\_\_\_ Elevation \_\_\_\_\_

Nat. veg. or crop \_\_\_\_\_ Climate \_\_\_\_\_

Parent material \_\_\_\_\_ Physiography \_\_\_\_\_

Relief \_\_\_\_\_ % Slope \_\_\_\_\_ Erosion potential \_\_\_\_\_

Drainage \_\_\_\_\_ Depth to water table \_\_\_\_\_ Est. permeability \_\_\_\_\_

Soil moisture (estimated) \_\_\_\_\_ Root distribution \_\_\_\_\_

Comments-Remarks: eg. presence of pans, concretions

| <u>Horizon</u>  | <u>Depth</u> | <u>Thickness</u> | <u>Boundary</u> | <u>Dry/Moist</u> | <u>Texture</u> | <u>Structure</u> |
|-----------------|--------------|------------------|-----------------|------------------|----------------|------------------|
| A <sub>00</sub> | +1cm.        | 1cm.             | even            | 4YR              | (litter)       | _____            |
| :               | :            | :                | :               | :                | :              | :                |
| :               | :            | :                | :               | :                | :              | :                |

- (a) United States Department of Agriculture, Soil Survey Manual, Handbook No. 18, Agricultural Resource Administration, Bureau of Plant Industry, Soils and Agricultural Engineering. U.S. Government Printing Office, Washington, D.C., 1951, pp. 138-140.



mean is estimated, can be obtained.<sup>4</sup> The soil samples (as well as plant material and water samples) being prepared for analysis should be selected in a random fashion to eliminate the problems associated with systematic analytical error.



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## APPENDIX A7.2

### SOILS--CHEMICAL ANALYSIS PROCEDURES

#### Preparation for Analysis--Total Trace Ion Content

The following procedure will be applied to all soil materials chemically analyzed for the designated trace element suite; preparatory methods prior to other analytical procedures will be given in the appropriate subsection of this appendix.

1. The sample bags will be removed at random from cold storage and allowed to come to room temperature. Approximately 100 gms of soil will be removed (per quadrat surface sample set) from each selected macroplot sample, then mixed, heaped, and quartered on a clean Teflon sheet. Two of the quarters will be transferred to a 250 to 400 ml labeled beaker, then air dried at  $40 \pm 2^{\circ}\text{C}$  in a forced-draft oven for 26 to 36 hours. The drying process will be repeated for the subsurface samples starting with 60 to 80 gms per sample bag.

2. The dry samples will be ground individually in a 250 to 500 ml capacity mortar with a pestle (to disrupt any existing soil structure but not interfere with soil texture). The material then will be sieved through a 2 mm (aperture) stainless steel screen; the trapped gravel-stone fraction per sample will be discarded. The passed volume then will be reground in a ceramic mill to pass a 100-mesh stainless steel screen and split (e.g., via a Humboldt Model H-3980 apparatus) into two subsamples. Both subsamples per bag will be stored in individual, labeled plastic or glass vials (100 ml capacity, with seals) at  $\sim 0^{\circ}\text{C}$  until analysis.

3. Five grams (to the nearest 0.1 mg) will be removed from each of the thawed samples (the first vial from each set). Each 5 gm aliquot will be



placed into a 500 ml Erlenmeyer flask and digested in the manner described in Appendix A6.2; the instrumental step will utilize either a research-grade, carbon-cup atomic absorption or inductively coupled Argon plasma unit.

#### Preparation and Analysis of Bioavailable Trace Ion Levels

Preparation and analysis of bioavailable trace ion levels will be accomplished in the following manner:

1. About 10 grams (weighed to the nearest 0.1 mg) of the dried, ground sample from each vial will be weighed out into individual, 125 ml widemouth Erlenmeyer flasks.
2. About  $20 \pm 1$  ml of DTPA extraction solution (composed of  $5 \times 10^{-3}$ M diethylene triamine pentaacetic acid,  $10^{-2}$ M anhydrous  $\text{CaCl}_2$  and  $10^{-1}$ M triethanolamine, adjusted to a pH of 7.30) will be added to each sample, slurried, and then shaken intermittently for two hours.
3. The extracts and reagent blanks will be filtered through Whatman No. 4 filter paper under light suction. The filtrates will be collected in 25 ml volumetric flasks, diluted to volume, and stoppered.
4. The samples then should be analyzed by the above type instrumental methods as quickly as possible. If estimated standing time is more than two hours, the flasks should be stored at  $5 \pm 1^\circ\text{C}$  until analysis; this cool storage should minimize potential "negative" errors arising from biological incorporation away from the soil extract solution prior to chemical analysis.

#### Determination of Soil pH

Soil pH will be determined as follows:

1. A 5-gram portion (weighed to the nearest 0.01 gm) from the first vial per sample will be transferred to a 150 ml beaker, and a 1:2 (w/v) soil: DI-water slurry will be prepared.



2. The slurry will be stirred/swirled gently to assure homogeneity and then allowed to stand covered for 30 minutes. The pH of the slurry will be determined while stirring with a pH meter previously calibrated at pH's of 4.00 and 7.00.

#### Determination of Soil Moisture Content

Determination of soil moisture content will be accomplished in the following manner.

1. A 5-gram portion of the initial undried but screened sample bulk per collection bag will be placed in a tared weighing vial ( $\approx$  30 ml capacity) and weighed immediately. The sample vials may be stored temporarily before weighing if evaporation losses are evaluated to be insignificantly small.
2. Each sample will be placed in a drying oven set at  $100 \pm 2^{\circ}\text{C}$  with the vial's lid off and dried to a constant weight. After a 24-hour period, the samples will be removed from the oven, each cover set ajar, and the samples stored in a desiccator (containing magnesium perchlorate or calcium sulfate) until cool.
3. The covers will be replaced fully, then weighed to the nearest 0.1 mg to a constant weight. The percentage of hygroscopic water per sample, as expressed on a dry weight basis, can be computed from the following formula:

$$1 - \left( \frac{\text{weight of wet soil}}{\text{weight of dry soil}} \right) \times 100$$

#### Discussion

The determination of total (acid-extractable) levels of the chosen trace element suite will be emphasized in the initial phase of the Water Quality/Biological Monitoring and Evaluation Program (WQ/B) reconnaissance until detailed data on both trace element stack emission characteristics and airflow modeling become available. Such information will allow reordering of trace element analysis priorities as well as more precise location of



both terrestrial and aquatic monitoring stations. The trace element terrestrial monitoring thus will focus initially around Lundegardh's "triple analysis" (as translated in Goodall and Gregory<sup>1</sup>), namely, analysis of plant tissue as well as surface and subsurface soils per given site.

In the drying of soil samples, temperatures much  $> 45^{\circ}\text{C}$  can alter the exchange characteristics of the clay-organic colloid fraction of the soil, thus biasing the estimated cation exchange properties<sup>2</sup> and the consequent estimate of its effect on the bioavailability of a given trace ion at a given site; temperatures much greater than  $40^{\circ}\text{C}$  also could induce losses of volatile trace elements, as discussed in the case of plant selenium analysis by J. H. Watkinson.<sup>3</sup> A stainless steel screen is reasonably inert to potential contamination of the samples at this stage of processing; the procedure for sieving the soil materials is similar to that used by Goodman and Roberts in their study of nonferrous smelter impact in the Swansea Valley, Wales.<sup>4</sup>

As proposed in the September 1977 progress report,<sup>5</sup> the trace element monitoring program eventually will diversify from reconnaissance into consideration and analysis of trace element biogeochemical cycling. Such a study can be initiated once a sufficient ( $\approx$  two years data) amount of baseline-background information has been gathered and statistically interpreted. A comprehensive study would require the periodic measurement of a host of soil parameters (discussed below) per site. At this time, the WQ/B personnel could begin to separate the various factors involved in ecosystem dynamics from the potentially adverse effects of the CDIF trace element emissions on the given biological community.

An estimate of bioavailability changes throughout a given soil profile for the suite of target trace elements will be accomplished by the DTPA



extraction procedure; this method<sup>6</sup> should produce more reliable, accurate results than if the corresponding metal ion-EDTA complexes were utilized. The data collected also should be in a form comparable to that being gathered by F. F. Munshower, et al.<sup>7,8</sup> in the vicinity of the nearly pristine Colstrip, Montana area.

M. L. Jackson has stated that the observed pH of a soil sample taken at field (moisture) capacity is probably the most valid estimate of hydrogen ion activity at a given site. However, it also has been noted that certain soil reactions are hastened by drying, thus allowing the soil(s) to come more nearly to chemical equilibrium.<sup>9</sup> Over the range from the "sticky point" (i.e., 2:1 w/v soil:water ratio) to "solution" (1:5 w/v soil:water ratio), the change in the pH of a given sample may be as much as one whole unit.<sup>10</sup> Michael Peech has stated that soil pH, measured using  $10^{-2}$ M  $\text{CaCl}_2$  as solvent, is approximately independent of dilution over a wide range of soil:solvent ratios.<sup>11</sup> He also observed that the pH of a 1:2 w/v soil:calcium chloride solution slurry was about 0.5 pH unit lower than when water was the solvent. Although the  $10^{-2}$ M  $\text{CaCl}_2$  solution would moderate measurement errors resulting from the junction potential effect, it also would mask intersite variability of the soil's salt content. The  $\text{CaCl}_2$  solution's unwarranted buffer properties would bias the resulting pH observation per sample, thus desensitizing this factor in the analysis of general trace ion bioavailability.

The gravimetric determination of soil moisture content is modeled after the method given by W. H. Gardner.<sup>12</sup> The application of this approach is intended for use in routine work where moderate precision (e.g.,  $\pm 0.5$  percent variability) is desired; it was suggested by the author to at least run duplicates for the given samples. The observed water content values for stony or gravelly soils, on either a mass or volume basis, can be grossly



misleading. "This arises from the fact that a large rock can occupy appreciable volume in a soil sample and contribute appreciably to the mass without making a commensurate contribution to the porosity or water capacity of the soil" (p. 94).<sup>12</sup> Thus, it is important to express the results in terms of the particle size fraction analyzed (in this case, that which can pass a 2-mm mesh screen).

The future consideration of trace element biogeochemical cycling will involve determinations of the following physical and chemical parameters (listed with possible methods of analysis):

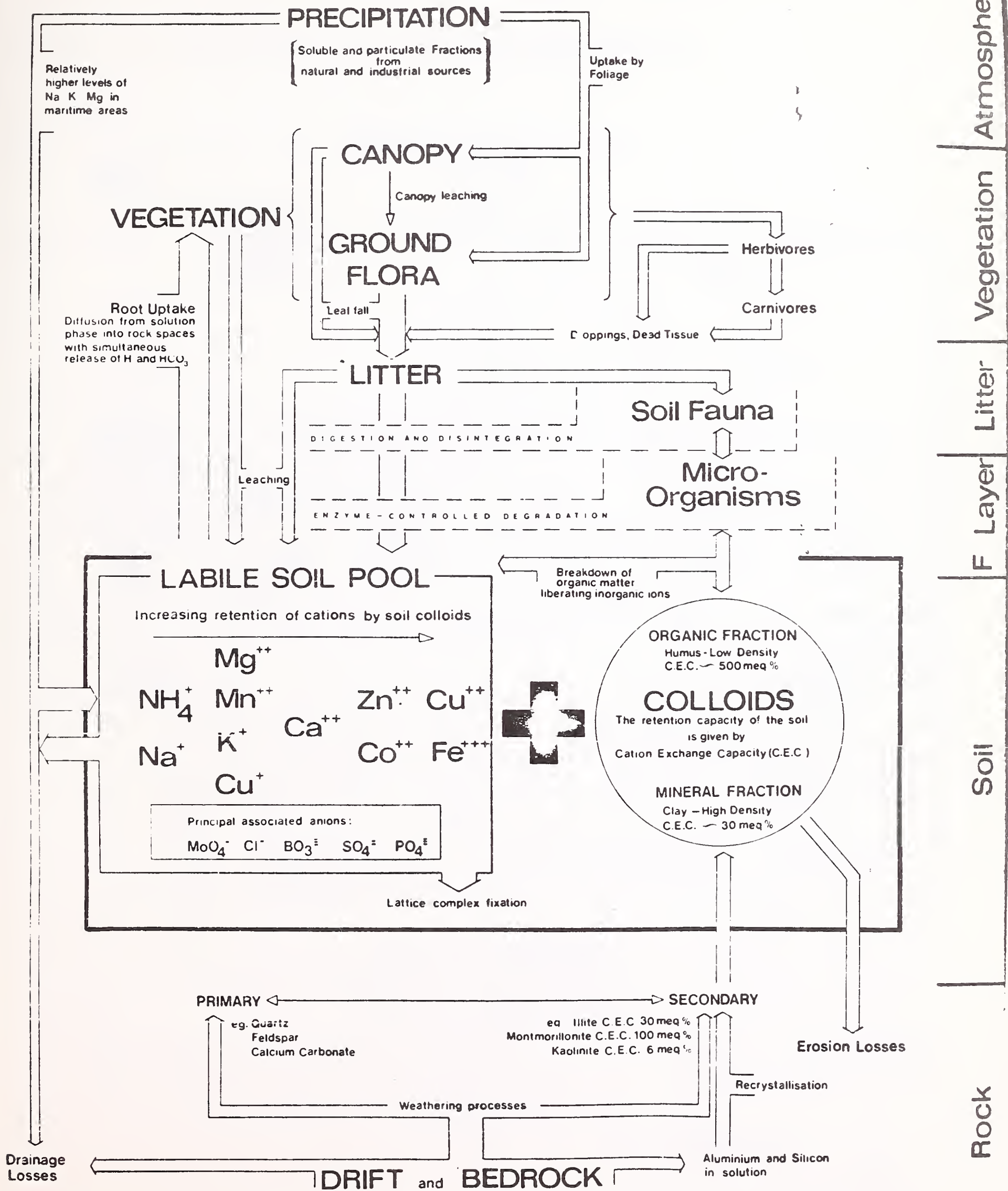
1. Physiochemical characterization of the organic matter soil fraction,<sup>13,14</sup>
2. Total nitrogen content,<sup>15</sup>
3. Cation exchange capacity,<sup>16</sup>
4. Allophane-allophanic clay(s),<sup>17</sup>
5. Particle fractionation (quantification of soil texture),<sup>18</sup> and
6. Redox potential<sup>19</sup> ( $E_h$ ).

The first two categories of the above list represent an attempt to grossly define the various organic molecular components that influence the general cycling of ions through a given soil profile (Figures A7.2-1 and A7.2-2). Detailed influences on these cyclical processes can be studied through knowledge of the general chelating capacity parameters, 3 and 4, and particle size fractionation (which contributes to both aeration effects and porosity-soil permeability characteristics). Finally, the correlation of redox potential data with pH data from a given site will allow the construction of ionic-stability diagrams (e.g., for selenium)<sup>20,21</sup> or for estimation of bioavailable levels of the target trace elements from total, acid-extractable data.<sup>22</sup> In the assumed well-aerated soil systems surrounding the CDIF site, the usually low concentrations of redox couples (e.g.,  $Fe^{+2} - Fe^{+3}$ ) reduces



Figure A7.2-1

A Simplified Outline of the Circulation of Mineral Nutrients in the Terrestrial Ecosystem<sup>(a)</sup>



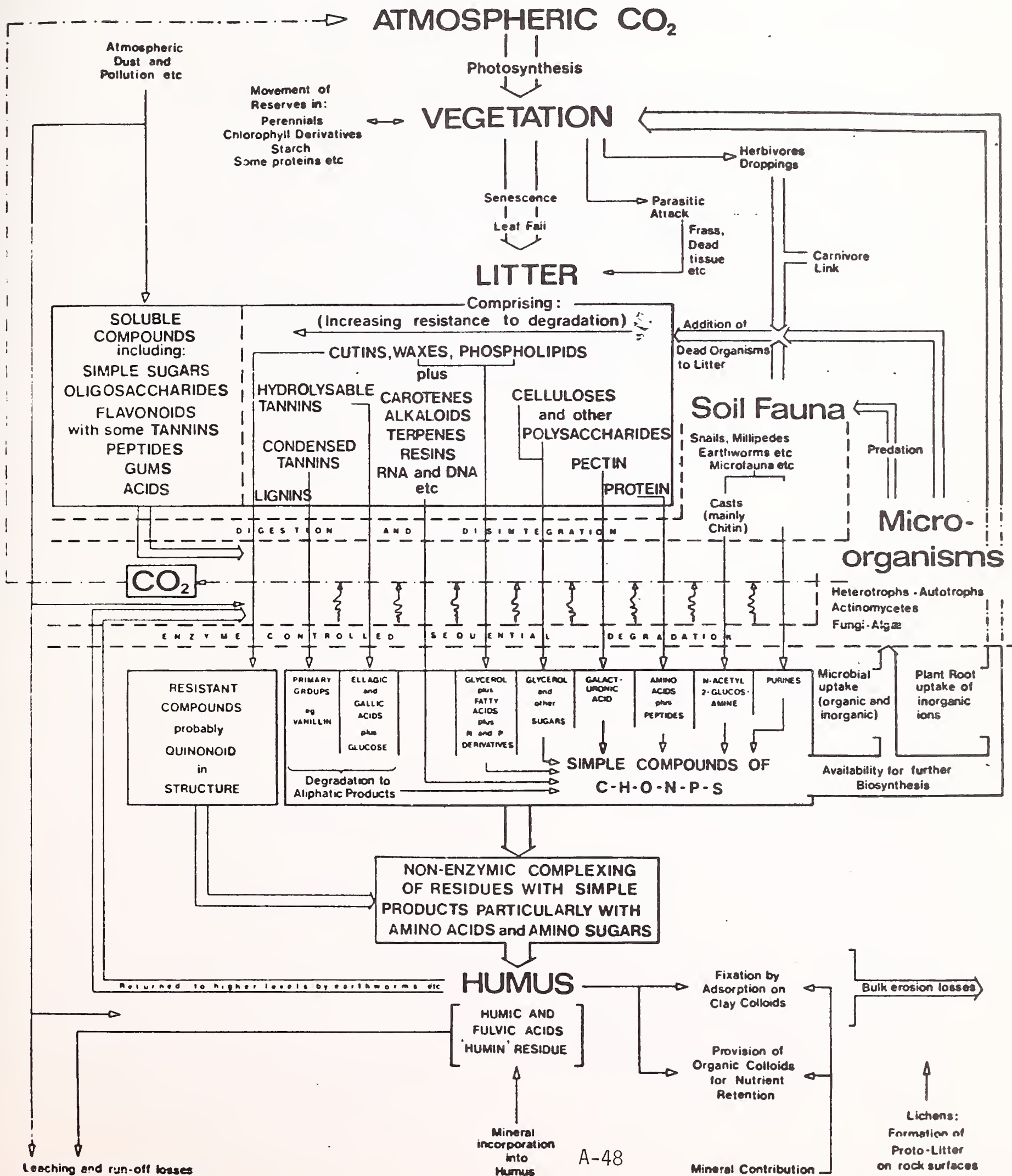
(a) Allen, Stewart E, Chemical Analysis of Ecological Materials, Halsted Press, John Wiley and Sons, 1974.



Figure A7.2-2

A Simplified Outline of the Carbon Circulation Scheme in the Terrestrial Ecosystem<sup>(a)</sup>

(a) S. E. Allen, Chemical Analysis of Ecological Materials.





the stability, reproducibility, and general usefulness of the redox determination;<sup>23,24</sup> thus, the above bioavailability extrapolations must be interpreted carefully and critically. It is suggested that several tests for  $E_h$  be run throughout the growing season (e.g., at the same time as for the "cool" and "warm" grass species biomass sampling periods).

The influence of the soil microbial population<sup>25,26</sup> on trace ion bioavailability must be known if a complete interpretation of plant assimilation-absorption processes is to be determined. Thus, a biological characterization of the soil microflora should be initiated, as suggested by William Barry.<sup>27</sup> Microbiological culture and counting techniques for the various life forms (e.g., algae, fungi, the microfauna, nematodes, and microarthropods) can be found in C. A. Black's test of microbiological methods (pp. 1452-1535).<sup>28</sup>



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## APPENDIX B



## APPENDIX B1

### AQUATIC SAMPLING--MICROBIOLOGICAL ANALYSIS

#### A. Membrane Filter (MF) Fecal Coliform

At each site/sampling period (monthly), the samples will be iced after collection in the field and analyzed by the standard membrane filter technique as described in the 14th edition of the APHA, et al., Standard Methods.<sup>1</sup> Approximately every 13th sample will be duplicated and, in an effort to assure quality control, prepared so the procedures and recovery rates can be checked. Presently, plate counts are being accomplished with a zoom-capacity stereoscope utilizing color plate identification aids from Millipore.<sup>2</sup>

#### Discussion

Fecal coliform examinations differentiate the total coliform group from those that originate in the gastrointestinal tract of mammals. These latter forms are general indicators of domestic contamination of a water body (e.g., through improper treatment or disposal of domestic sewage).

In all microbiological work, standard sampling techniques<sup>1,2</sup> and sterile laboratory techniques<sup>3,4,5,6</sup> will be followed; media preparation by compounding prepared, commercially available reagents from recognized dealers will continue to be utilized.

#### B. MF Total Coliform

The determination of the levels of this group in a given sample volume (ranging from 100 ml to 1 ml) will rely on the APHA et al's MF-Endonutrient procedure (see citation 1, Section A); this method is more sensitive, but less selective, than the above fecal coliform test.



## Discussion

In addition to the fecal types discussed in Section A, the coliform group includes a number of free-living, heterotrophic genera that largely reside in the upper horizons of given soil profiles and/or benthic sediments. Thus, a disturbance of such habitats by logging, road construction, or stream dredging/rechannelization could induce (through site runoff) elevated total coliform counts in an adjacent body of standing or running water.

An increase in coliform counts (per unit sample volume), in combination with physiochemical data (e.g., nitrate levels), would allow the staff personnel to better assess and locate the sources of adverse impact on the given body of surface or subsurface water.

### C. MF Total Growth Potential on Nutrient Broth

Utilizing a nonspecific nutrient medium, one can assess the potential total, diverse, microbial community present in a given water sample (as collected on a membrane filter). The evaluation of such changes in microbial levels over a period of time allows assessment of the relative degradation of a given body of surface or subsurface water (e.g., through an introduction of "foreign" nutrients into a given water source, a possible change in the hydrogeologic source of a given body of subsurface water, or its contamination by urban or domestic effluents).

### D. Fungi and Detrivores

Presently, the necessity and feasibility of mycologic (fungi) examination of waters from the aquatic sites is under assessment. If such analysis is determined to be warranted, recognized procedures/techniques as given in Standard Methods will be implemented (or developed if necessary) to facilitate this phase of the microbiological study. The detrivore trophic



level of any given aquatic ecosystem may be of use either as a) indicator(s) of relative community health or b) critical concentrators of trace ions (especially those possibly released during the course of MHD-related operations).

E. Other

This category could include, for example, the determination of a given sample's fecal coliform/fecal streptococcus ratio. This would be useful in determining the probable bacteriologic pollution source. A ratio  $>2$  would imply human origin, while ratios  $<1$  tend to arise from the gastrointestinal tracts of livestock.

As the study's literature review and data analysis progresses, this section could be expanded to include any of the diverse forms of life known to be present in ecosystems similar to those existing in the CDIF study area. This subsection would be used to fill any vacancy evaluated to be worthy of scientific study in the course of the aquatic monitoring program of the WQ/B.

The microbiological phase of the baseline aquatic monitoring program will aid in the overall documentation of changes in the study area's water quality over a period of time. In agreement with the trace ion emphasis of the WQ/B, the monitoring of trace ion levels in microbial populations could serve as a means of quantifying changes in trace element "loading" of aquatic ecosystems and possibly in the identification of their ultimate sources (e.g., domestic sewage or coal pile runoff).



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## APPENDIX B2

### PERIPHYTON ANALYSIS: 4CM<sup>2</sup> SCRAPE

The periphyton communities in the given area's aquatic environments are being identified via Barry's<sup>1,2</sup> four-cm<sup>2</sup> scrape method, as documented in McNabb<sup>3</sup> and Prescott.<sup>4</sup> This technique utilizes a soft, plastic template that is placed on an initially submerged object (e.g., rock or log) and the sampling area scored (to delineate its boundaries) with a scalpel or sharpened spatula. The field technician then carefully removes the total surface growth from the defined area, transferring it to a clean, empty vial. The periphyton sample is preserved by adding a transeaux solution<sup>2,4</sup> adapted for field use and returned to the laboratory for taxonomic identification<sup>4,5,6,7,8</sup> and counting.<sup>8,9</sup>

#### Discussion

This particular method will continue to be utilized as a check on the proposed procedure in Subsection B, below. Identification of the indigenous, lenitic periphyton populations has been judged to be more responsive/representative of on-site water quality than the more transient plankton community.



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## APPENDIX B3

### PERIPHYTON ANALYSIS: ARTIFICIAL SUBSTRATES

#### A. Standard (Glass Slide) Method

Clean glass slides 25 x 75 mm (1" x 3") will be exposed to the aquatic environment using a variation of the Aufwuchs sampling described in Sládečková;<sup>1</sup> sampling will be accomplished on a quarterly (calendar year) basis. Standard methods of inducing periphyton colonization on artificial structures will be utilized.<sup>1,2,3,4,5,6</sup>

#### Discussion

The periphyton that colonize an artificial surface are usually those present in the area. Comparison of the data and literature available shows fair to good representation of the native community if exposure is lengthy enough and clean, non-reactive materials are used.

#### B. Experimental (MF) Method

In addition to the method above, an experimental phase utilizing a Millipore<sup>7</sup> membrane filter (MF) will be employed. After a yet to be determined length of time, the MF will be removed and the resident, indigenous populations will be fixed (using three to four drops of transeaux solution) and transported to the laboratory.

At the laboratory, the filter(s) colonized by the periphyton or Aufwuchs community will be flooded initially with immersion oil (NRI=1:515) and then dried. The desiccated filter will be treated with several additional drops of the above oil and placed on a clean, dry 5 x 15 cm glass or petri slide. A clean, dry cover slip then will be applied; the viewing area will be "debubbled" and finally sealed with Permunt or an equivalent sealing solution.<sup>7</sup>



## Discussion

The nearly identical refractive indices of the filter and oil allow ready identification/quantification of the community attached to the MF. The resulting observations will be compared with the data gathered by methods described in Appendices B2 and B3(A).

In this manner, a rapid, convenient, and economical technique for the classification/quantification of the area's periphyton communities may be developed, documented, and disseminated to the interested scientific audience in this field.

### C. Productivity

Estimated levels of net primary productivity within the periphyton community can be extrapolated from a quantitative chlorophyll determination. It is proposed that duplicate slides be used from methods A and B, above, utilizing the scraping/acetone extraction method of Stein<sup>8</sup> and Lind<sup>9</sup> in the former case, while including the MF as part of the analyte in the latter case. The determining step will be performed on the WQ/B's Perkin-Elmer Model 550 uv/vis spectrophotometer.

## Discussion

The establishment and documentation of baseline and continuing chlorophyll/taxonomic data would allow evaluation and valid interpretation of changes in a given aquatic community's structure and function. Such information could be correlated with potential MHD CDIF trace element impacts.

The Aufwuchs communities will not have established a dynamic equilibrium with the stream's environment.<sup>10</sup> By the examination of such populations on a regular basis (e.g., three-week intervals), relative population balances can be established for these artificial substrate community types.<sup>11,12</sup>



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APPENDIX B4  
PERIPHYTON--TRACE ELEMENT ANALYSIS

A. Random Collection Method

The staff field personnel have taken replicate random samples on a seasonal basis of the conspicuous macroscopic fraction of each site's attached algal community. At each site, the method involves the following steps:

1. The first vial per set will contain a detached specimen, transferred to a 30 ml glass vial by tweezers or a spatula, then preserved in transeaux solution. The second vial will contain the same specimen type (species) but contained in stream water. All samples are stored in ice prior to transport back to the laboratory.
2. The first (transeaux) sample then will be identified as to species, while the latter will be air-dried at  $40 \pm 2^{\circ}\text{C}$  and digested as described in Appendix A6.2. Trace element analysis will be accomplished by either carbon-cup atomic absorption spectrophotometry or inductively coupled Argon plasma emission spectroscopic methods.

Discussion

The inherent problem of this method is the assurance of near physiologic duplication within each replicate sample set or the representativeness of a given area's algal community in terms of response to potential environmental insult. However, accumulated field/lab experience should allow the evaluation of the validity of this method within the first year or so of research effort.

The long-term evaluation of the above data will allow a quantification of any potential MHD CDIF trace ion emission/effluent release



effects on the study area's aquatic periphyton. Presently, the following areas are being researched: a) the ability of particular algal species to actively accumulate trace ion concentrations up to an order of magnitude greater than levels of physiologic need; and b) the influence of given trace ions on growth/reproduction rates or the inhibition of particular catabolic/anabolic pathways within particular algal species. A detailed presentation of the results of this survey will be discussed in a future report.

B. Membrane Filter--Attached Algae Chemical Analysis

A replicate membrane filter sample from Appendix B3(B) methodology will be air-dried at  $40 \pm 2^{\circ}\text{C}$ , weighed to the nearest 0.1 mg and digested overnight at room temperature in a covered 150 ml beaker containing  $\approx 10$  ml of mercury-grade, concentrated  $\text{HNO}_3$ . The resulting solution then will be divided into two process streams:

1. For analysis of volatile trace ions, e.g., Se, Hg, by appropriate atomic absorption-type methods,<sup>1,2,3</sup> and
2. For analysis of the non-volatile fraction by appropriate atomic absorption-type methods.<sup>1,3</sup>

Discussion

The validity of this method of analysis of trace ion concentrations in known periphyton populations is limited by inherent errors in sample preparation and the given sensitivity of the instrumental methods employed in the determinative step.



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APPENDIX B5  
AQUATIC VASCULAR PLANT STUDIES

With the exception of groundwater/well sites, representative vascular species from the aquatic sites will be inventoried and subjected to simple vegetational analysis (e.g., relative abundance) schemes. Samples of the flora will be collected and stored in ice for transport back to the laboratory. The analytical procedures will follow those described in the terrestrial Appendix A6.2.

The long-term evaluation of this data, when correlated to that data obtained in Appendices B2 through B4, should allow estimation of elevated levels of trace ions in the area's vascular flora resulting from potential MHD CDIF trace ion emissions.



## APPENDIX B6

### MACROINVERTEBRATE AND VERTEBRATE COLLECTIONS

The present emphasis on the primary trophic level (i.e., in terms of characterizing their structure and function as well as baseline trace element loading) precludes analysis of "higher" herbivore/carnivore systems. However, documented elevation of trace ion concentrations and/or significant changes in community types at the producer level will necessitate a more detailed hierarchical ecological analysis of the given impacted community. In the anticipation of such an event, WQ/B personnel will collect, identify, and catalog these specimen types, when available, for future analysis and correlation to the vegetational data.



APPENDIX B7  
AQUATIC CHEMICAL ANALYSIS

At each site, separate volumes of water will be collected. These include a one liter sample to be acidified with 5 ml of concentrated, re-distilled (mercury grade)  $\text{HNO}_3$  to a  $\text{pH} \leq 2$ , then capped and swirled and a two liter nonacidified sample (i.e.,  $5 \leq \text{pH} \leq 8$ ). The first aliquot will be utilized for trace element analysis, as described in Appendix A6.2; the nonacidified aliquot will be used to measure the following water quality parameters:

- pH, to the nearest 0.1 unit (in the laboratory),
- dissolved oxygen,
- ammonia,
- ortho-phosphate,
- total hardness,
- calcium hardness,
- magnesium hardness,
- chlorides,
- total solids (total residue),
- dissolved solids (filterable residue),
- suspended solids (non-filterable residue),
- turbidity, (in JT units by the Formazin method),
- total iron,
- total alkalinity (as  $\text{CaCO}_3$ ),
- hydroxide alkalinity,
- bicarbonate alkalinity, and
- carbonate alkalinity.



All of the above parameters will be determined by standard methods;<sup>1,2,3,4</sup> with the exception of pH and turbidity, analytical accuracy will be set at the nearest 0.1 of a mg/l. Furthermore, these additional parameters will be measured by standard methods<sup>1-4</sup> in the field:

- temperature, to the nearest 0.5<sup>0</sup>C,
- conductivity, in  $\mu$ mhos/cm,
- pH, to the nearest 0.5 unit, and
- flow rate, in m<sup>3</sup>/sec.<sup>5</sup>

#### Discussion

The above standard water quality parameters are being monitored to establish a water quality baseline for the CDIF study area. Quantitative changes in the water quality thus can be documented with definition and identification of the causal agent. Contingent upon the recognition of the causal agent(s), regardless of its source, the WQ/B staff will recommend mitigating measures to the appropriate authorities or persons.



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## APPENDIX C



## APPENDIX C

### COMMENTS ON DATA ANALYSIS

For each of the described environmental parameters, the descriptive population parameters ( $\mu$ ,  $\sigma^2$ ,  $\sigma$ ) will be estimated from the sample statistics within the given 95 percent confidence interval. The standard error (e.g.,  $s_m - s_x/\sqrt{n}$ ), an index of measurement error variability from one subplot to another, also will be computed for each variable.

Graphic presentation formats<sup>1</sup> will include, for example

- multiple tracing/composite histograms of  $[M^{+n}]$ 's between surface and subsurface soil levels at a given exclosure,
- mirror-image line graphs of annual cycling of a given algal species abundance at an aquatic site, and
- scatter diagrams showing percent cover per quadrat between exclosures and (changes) over time.

In addition, single and multiple correlation coefficients and their level of significance will be calculated between an abiotic factor (e.g., trace ion level) and biotic factor (e.g., biomass) measured in this study. Tentatively, "least-squares" linear or curvilinear product moment techniques will be applied<sup>2,3,4</sup> to such parameters. The independent variable (e.g., Pb levels) must be normalized with common variance against the dependent variable (e.g., biomass) if this statistical technique can be applied validly. The results of the correlation and regression calculations also will be shown graphically via scatter diagrams. It might be of interest to apply power-efficient, non-parametric techniques<sup>5</sup> to the above statistical considerations since the assumptions necessary for correct application are less rigorous than for the parametric equivalent tests.



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